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Stimulation of α_1-adrenoceptors in rat kidney mediates increased inositol phospholipid hydrolysis

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1 The molecular events which follow activation of α_1-adrenoceptors in rat kidney were investigated by measuring inositol phospholipid hydrolysis. Slices were labelled with [^{3}H]-inositol (0.25 μM) and the accumulation of [^{3}H]-inositol phosphates ([^{3}H]-IP's) was measured after stimulation with α-adrenoceptor agonists.

2 Phospholipid labelling was both time- and Ca^{2+}-dependent. In kidney, Ca^{2+} (1 mM) increased the incorporation of [^{3}H]-inositol by 49% and in cerebral cortex reduced it by 46%.

3 Following addition of noradrenaline (NA, 1 mM), accumulation of [^{3}H]-IP's increased linearly for at least 60 min. In Ca^{2+}-free buffers a 2.1 fold increase in [^{3}H]-IP accumulation was observed and further increases in stimulated and control levels were produced in the presence of Ca^{2+} (2.5 mM). These responses were attenuated by the inclusion of indomethacin (10 μM) and abolished in the presence of EGTA (0.5 mM). Responses to (−)-NA were more than 4 fold higher in the renal cortex than in the medulla.

4 Separation of the IP's which accumulate after α-adrenoceptor agonists showed that after 60 min stimulation the major products were glycerophosphoinositol and inositol-phosphate with smaller amounts of inositol-bisphosphate and inositol-trisphosphate.

5 The most effective agonists tested for stimulation of accumulation of [^{3}H]-IP's were (−)-NA > phenylephrine > methoxamine, (+)-NA. Clonidine and (−)-isoprenaline were ineffective at concentrations up to 100 μM. The order of effectiveness of α-adrenoceptor antagonists was prazosin > BE2254 > phentolamine > idazoxan > rauwolscine.

6 The results indicate that α_1-adrenoceptors in rat kidney are linked to phosphoinositide hydrolysis and that this response is localized mainly to the renal cortex.

Introduction

The existence of two pharmacologically distinct subtypes of α-adrenoceptors based upon the relative affinities of a series of agonists and antagonists is well established (Starke, 1981). However, α-adrenoceptor subclassification extends further than differences in the drug recognition site since activation of each receptor subtype also leads to separate intracellular events (Fain & Garcia-Sainz, 1980). In most cases α_2-adrenoceptors are coupled to adenylate cyclase by a guanine nucleotide binding regulatory protein (N_i) and activation leads to decreased activity of the enzyme and a subsequent decrease in cyclic AMP production by the cell (Bylund & U'Prichard, 1983). On the other hand, activation of α_1-adrenoceptors leads to alterations in intracellular free Ca^{2+} secondary to hydrolysis of membrane phosphoinositides (Berridge, 1984).

Both α_1- and α_2-adrenoceptors have been demonstrated in rat kidney in radioligand binding studies (Woodcock & Johnston, 1982; Bylund & U'Prichard, 1983; Summers, 1984). α_2-Adrenoceptors have been shown to be functionally relevant in that they are coupled to adenylate cyclase (Woodcock & Johnston, 1982; Chabardes *et al.*, 1984) while stimulation of α_1-adrenoceptors mediates enhanced prostanoid release (Cooper & Malik, 1985), gluconeogenesis (Kessar & Saggerson, 1980; McPherson & Summers, 1982), renal vasoconstriction (Schmitz *et al.*, 1981; Smyth *et al.*, 1984; Cooper & Malik, 1985) and inhibition of the release of renin (Matsumura *et al.*, 1985). Sodium reabsorption is stimulated following administration of α_1-adrenoceptor agonists in rabbit (Hesse & Johns, 1984) and dog (Osborn *et al.*, 1983) kidney and is

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known to compete with gluconeogenesis for available oxidative energy in kidneys of steroid-treated rats (Silva *et al.*, 1980). However, little is known of the events that occur between the activation of renal α_1-adrenoceptors and the wide variety of end-organ responses which follow.

In brain (Brown *et al.*, 1984; Minneman & Johnson, 1984) and in many peripheral tissues including salivary glands (Michell, 1975; Berridge *et al.*, 1982), thyroid (Uzumaki *et al.*, 1982), smooth muscle (Villalobos-Molina *et al.*, 1982), adipocytes (Garcia-Sainz *et al.*, 1980), and liver (Harrington & Eichberg, 1983), activation of α_1-adrenoceptors is associated with increased phosphoinositide (PI) hydrolysis. The phosphorylated derivative of PI, phosphatidylinositol-4,5-bisphosphate (PIP_2) is hydrolysed to diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3) which are second messengers acting to stimulate protein kinase C and mobilize intracellular Ca^{2+} (Berridge, 1984; Nishizuka, 1984). Thus, PI hydrolysis has been proposed as the signal transducer for receptors which mediate cellular responses via changes in the levels of intracellular free Ca^{2+} (Michell, 1975; Berridge, 1984).

This paper uses a modification of the method of Berridge *et al.* (1982), to demonstrate that α_1-adrenoceptors in rat kidney are linked to PI hydrolysis.

Methods

Tissue preparation

Wistar rats (250–300 g of either sex) were decapitated, the kidneys removed and chilled in ice-cold modified Krebs-bicarbonate medium (composition in mM: NaCl 109, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, D-glucose 12, LiCl 10, EDTA 0.045, pH 7.4) previously gassed with carbogen (O_2/CO_2, 95:5, vol/vol). Slices of renal cortex or cerebral cortex (1 mm thick) were cut using a tissue slicer (Thomas), chopped into smaller slices (350 × 350 × 1000 μm) on a McIlwain's tissue chopper, and then washed and incubated at 37°C with one intermediate change of buffer and constant carbogen gassing for 30 min to increase ATP levels in tissues (Brown *et al.*, 1984).

Labelling of membrane phospholipids and measurement of production of [^{3}H]-inositol phosphates

Aliquots of kidney slices (50–125 μl) were transferred to 10 ml flat-bottomed tubes containing 165–240 μl of buffer, 10 μl of [^{3}H]-inositol (final concentration 0.25–0.28 μM) was added and the tubes were gassed with carbogen, capped and incubated at 37°C in a shaking water bath. After 30 min, agonists were added in a 10 μl volume, the tubes re-gassed and incubated for a further 60 min. Tissues were exposed to antagonists for 20 min before the addition of agonists. After stimulation, tissues were washed three times by resuspension in 3 ml ice-cold Krebs buffer before the addition of 0.94 ml of chloroform/methanol (1:2). After 15 min, chloroform (0.32 ml) and then water (0.32 ml) was added, the tubes vortexed and centrifuged (1000 *g*, 5 min) to aid phase separation. In some experiments, the incorporation of [^{3}H]-inositol into the phospholipids was measured by counting 200 μl samples of the lower chloroform phase following overnight drying. For the measurement of total [^{3}H]-inositol phosphates ([^{3}H]-IP's) a 750 μl volume of a 50% (w/v) slurry of Dowex-1 (100–200 mesh: × 8 in the formate form) resin stored in 0.1 M formic acid was added to glass columns. The columns were washed with distilled water (10 ml) and 900 μl of the upper aqueous phase applied. The columns were then washed with 5 mM myo-inositol (3 × 2 ml) and [^{3}H]-IP's eluted into scintillation vials by the addition of 1 M ammonium formate/0.1 M formic acid (2 × 500 μl) to the columns. Scintillation fluid (10 ml) was added and the radioactivity counted in a scintillation counter.

Separation of ^{3}H-labelled inositol phosphates

For separation and assay of the individual phosphorylated inositol derivatives, 300 μl of 1.0 M trichloroacetic acid (TCA) was added and the samples left to stand at room temperature for 15 min. After vortexing and centrifugation (3,500 *g*, 20 min), 500 μl aliquots of the supernatant from triplicate samples were pooled and the TCA removed by washing with 5 × 2 volumes of water-saturated diethyl ether; 3.5 ml of 5 mM $NaHCO_3$ was added to adjust the sample pH to between 7–8 and 4 ml of this extract was applied to columns containing 1 ml of Dowex resin and the phosphates separated by sequential elution with formate solutions of increasing strength. [^{3}H]-inositol was eluted with 15 ml of 5 mM myo-inositol. [^{3}H]-glycerophosphoinositol ([^{3}H]-GPI) was eluted with 30 ml of 5 mM sodium tetraborate/60 mM sodium formate. [^{3}H]-inositol-1-phosphate ([^{3}H]-IP_1), [^{3}H]-inositol-1,4-bisphosphate ([^{3}H]-IP_2) and [^{3}H]-inositol-1,4,5-trisphosphate ([^{3}H]-IP_3) were eluted with 20 ml of 0.2 M, 0.4 M and 1.0 M ammonium formate/0.1 M formic acid solutions, respectively. Eluates were collected in either 1 ml samples or in bulk in which case triplicate samples were taken from the bulk elution volume and the radioactivity corrected to obtain the total tritium eluted.

In experiments investigating the calcium requirement of PI hydrolysis, tissues were labelled with [^{3}H]-inositol for 1 h in Ca^{2+}-free Krebs buffer, then washed with 4 × 5 ml of the same buffer at 37°C. A fifth wash was performed in the appropriate buffer (Ca^{2+}-free, 2.5 mM Ca^{2+}, Ca^{2+}-free + 0.5 mM EGTA)

and aspirated to 300 μl whereupon 10 μl of noradrenaline (NA) or buffer was added and allowed to act for 60 min. This procedure was designed to dissociate the effects of Ca^{2+} on PI hydrolysis from its effects on labelling.

Analysis of results

Analysis of concentration-response curves was performed using the computer program, KA (McPherson *et al.*, 1983). This provides an iterative fit to concentration-response data and gives a best-fit estimate of EC_{50}. For antagonists, estimates of pA_2 values were obtained from the shift to the right of concentration-response curves produced by a single concentration of antagonist.

Drugs and chemicals

Drugs and chemicals used in this study were as follows: (−)-noradrenaline bitartrate, (+)-noradrenaline bitartrate, (−)-isoprenaline bitartrate (Sterling-Winthrop); prazosin hydrochloride (Pfizer); rauwolscine hydrochloride (Roth); phentolamine hydrochloride (Ciba-Geigy); idazoxan (Reckitt & Colman); indomethacin, phenylephrine hydrochloride (Sigma); methoxamine (Burroughs Wellcome); clonidine hydrochloride (Boehringer Ingelheim); BE2254 (2-[β-(4-hydroxyphenyl)-ethyl-aminomethyl]tetralone) (Beiersdorf); Dowex-1 (100–200 mesh: × 8, $-Cl^-$) resin (Sigma); [^{3}H]-inositol, sp.act. 15–20 Ci mmol^{-1} (Amersham) was purified before use on a Dowex-1 anion-exchange column. All other chemicals were of analytical grade.

Results

Incorporation of [^{3}H]-inositol into kidney slices

Incorporation of [^{3}H]-inositol into the phospholipid pools in brain is inhibited by Ca^{2+} (Kendall & Nahorski, 1984). These effects, which were clearly reproduced here, are in contrast to the effects seen in kidney where Ca^{2+} (0.03–1.0 mM) markedly enhanced labelling (Figure 1). In brain the addition of Ca^{2+} (1 mM) reduced incorporation by 46%, whereas in kidney Ca^{2+} (1 mM) produced a 49% increase in labelling (Table 1). Paradoxically, Ca^{2+} depletion with EGTA also tended to stimulate labelling in kidney although this effect did not reach significance. At all concentrations of Ca^{2+} tested, [^{3}H]-inositol incorporation was significantly higher in kidney than in brain.

Incorporation of [^{3}H]-inositol into kidney phospholipids was time-dependent reaching a plateau after 2 h (Figure 2). However, sufficient incorporation took

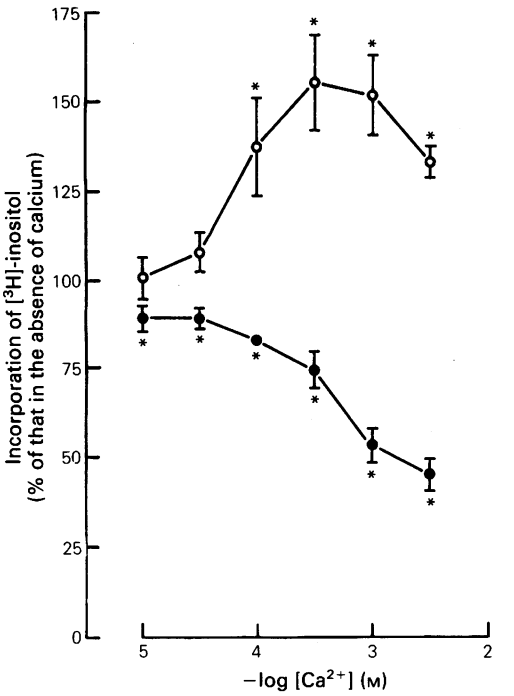

Figure 1 Effects of Ca^{2+} concentration on [^{3}H]-inositol incorporation in slices prepared from rat cerebral (●) and kidney cortex (○). Samples of the lower chloroform phase containing the [^{3}H]-inositol phospholipids were taken from slices preincubated in Ca^{2+}-free medium and then incubated in increasing concentrations of Ca^{2+} (10^{-5}–$10^{-2.5}$ M) for 90 min. Data expressed as a percentage of results in the absence of added Ca^{2+} represent the mean in three separate experiments conducted in triplicate; vertical lines show s.e.mean.
*Significant differences from slices incubated in the absence of added Ca^{2+} ($P < 0.05$, Student's t test).

Table 1 Effect of Ca^{2+} concentration on [^{3}H]-inositol incorporation in cerebral and renal cortical slices

	Cerebral cortex	*Kidney cortex*
EGTA 0.5 mM	67,370 ± 5,559	123,806 ± 15,200
No added Ca^{2+}	56,440 ± 3,552	109,566 ± 13,077
Ca^{2+} 1 mM	30,242 ± 3,880	162,854 ± 7,456

The figures given represent total [^{3}H]-inositol incorporation (d.p.m.) into 125 μl packed slices calculated from the amount of radioactivity present in a 200 μl sample of the lower chloroform phase. Values are mean ± s.e.mean for three separate experiments conducted in duplicate. Asterisks indicate where levels of incorporation are significantly different from those in the absence of added Ca^{2+} ($P < 0.05$, Student's t test).

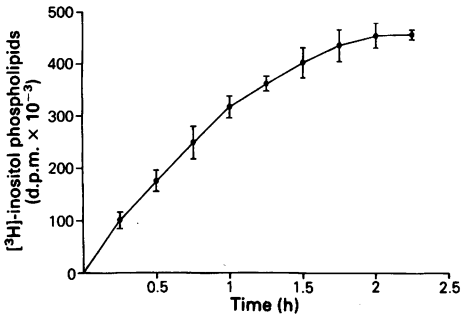

Figure 2 Time course of inositol phospholipid labelling in rat kidney cortical slices. Slices were incubated with [^{3}H]-inositol (0.28 μM) for various time periods, washed four times in ice-cold buffer and extracted as in methods. Samples of the lower chloroform phase containing the [^{3}H]-inositol phospholipids were taken in duplicate and counted. Data represent the mean of three separate experiments conducted in triplicate; vertical lines show s.e.mean.

place after 30 min to allow examination of drug effects on accumulation of IP's.

Accumulation of inositol phosphates after noradrenaline stimulation of kidney slices

Addition of noradrenaline (NA, 1 mM) to kidney slices in the presence of lithium resulted in an enhanced accumulation of inositol phosphates. Accumulation occurred in both control and stimulated slices but the rate of accumulation was higher in the stimulated slices (Figure 3). The linear rate of accumulation of IP's indicates that there is no desensitization of the NA response over this time period or significant degradation of NA. The presence of a Ca^{2+}-dependent turnover of IP's in the control slices may indicate the presence of locally produced agonists (Figures 3 and 5). A 60 min incubation period gave a clearly defined stimulation and was chosen for these experiments.

Separation of the IP's on ion exchange columns was achieved by use of buffers with increasing concentrations of ammonium formate (Figure 4a). After 60 min stimulation the major peaks were GPI and IP_1 with smaller amounts of IP_2 and IP_3 (Figure 4b). All peaks except IP_3 were markedly increased after stimulation by NA (10^{-3} M). The total radioactivity from control and stimulated slices for each peak is expressed as the histograms of Figure 4c, and show that the major products of stimulation are GPI and IP_1 with relatively small amounts of IP_2 and IP_3 indicating that under the conditions used here these have been dephosphorylated to IP_1. These experiments indicate that a significant fraction of the total radioactivity eluted with 1.0 M ammonium formate/0.1 M formic acid is

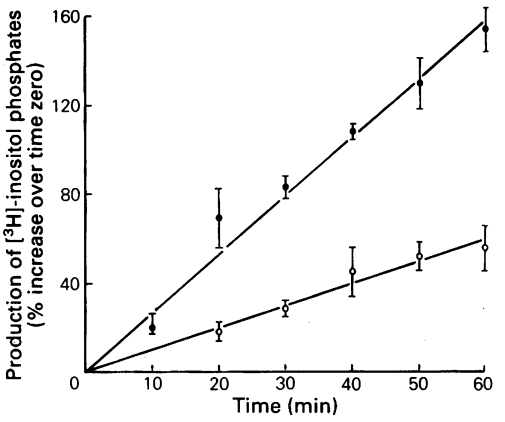

Figure 3 Time course for the production of [^{3}H]-inositol phosphates in rat kidney cortical slices. Tissues were labelled for 60 min with [^{3}H]-inositol, washed (4 × 3 ml buffer) and then incubated with or without noradrenaline (NA, 1 mM) for various time periods: basal (○); with NA (●). The reaction was stopped with the addition of chloroform/methanol. Data expressed as the percentage increase over time zero represent the mean of three separate experiments conducted in duplicate; vertical lines show s.e.mean.

[^{3}H]-GPI and that this product increases with stimulation. The role and significance of GPI is not clear at present.

The response to NA was Ca^{2+}-dependent since responses were abolished in the presence of EGTA (Figure 5). In the absence of added Ca^{2+}, NA increased PI hydrolysis to 214% of control, indicating that small concentrations of Ca^{2+} present in the tissue are sufficient for the response. Both NA-stimulated and control levels of [^{3}H]-IP's were increased in the presence of Ca^{2+} (2.5 mM) with adrenoceptor specific accumulation increased 232% compared to control. Addition of Ca^{2+} (2.5 mM) to tubes incubated with 0.5 mM EGTA restored full NA-stimulated responses indicating that the effects of EGTA are due to Ca^{2+} chelation.

Characterization and localization of the NA-stimulated accumulation of IP's in kidney

A selection of adrenoceptor agonists and antagonists was used to characterize the receptor involved in NA-induced accumulation of renal IP's. Concentration-response relationships for accumulation of [^{3}H]-IP's are shown in Figure 6. The maximum increase in response to NA was 214% of control. Stereoselectivity was observed for the isomers of NA with the (−)-isomer some 10 times more effective than the (+)-isomer. The order of potency of the agonists was (−)-

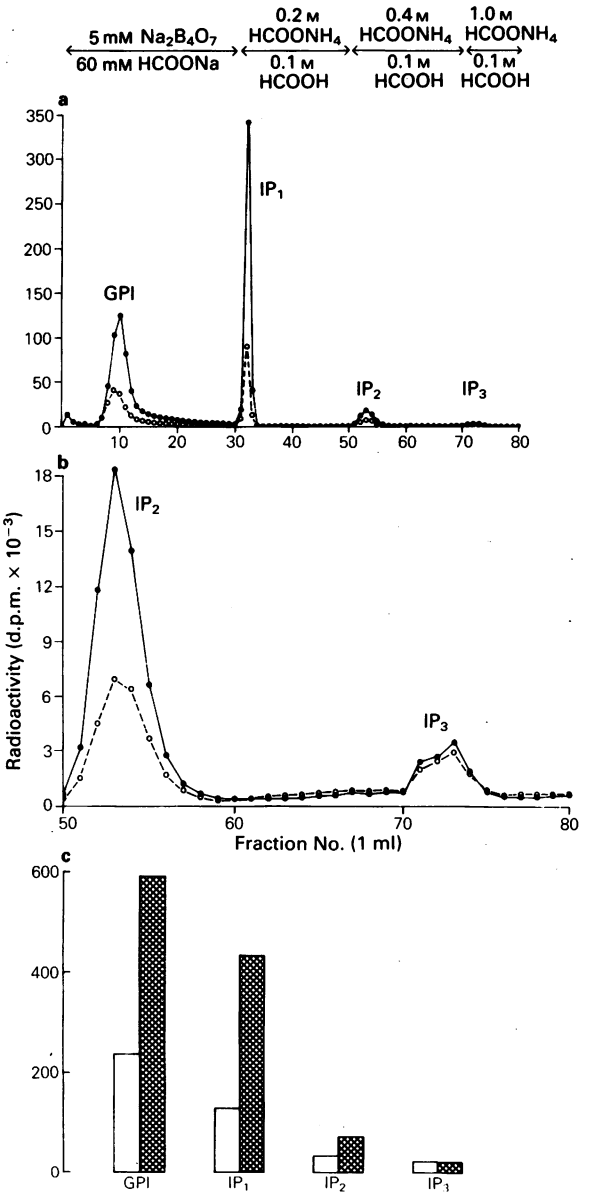

Figure 4 Anion exchange chromatography of [^{3}H]-inositol phosphates extracted from rat kidney cortical slices following stimulation with noradrenaline (60 min). The water soluble products obtained by TCA extraction of the tissues were applied to Dowex-1 anion exchange columns and eluted with formate solutions of increasing strength as described under methods. The elution profile in (a) shows radioactivity collected following application of 1 ml aliquots of formate solutions to the columns. Four peaks were identified and according to Berridge *et al.* (1982) correspond to glycerophosphoinositol (GPI), inositol-phosphate (IP_1), inositol-bisphosphate (IP_2) and inositol-triphosphate (IP_3). The [^{3}H]-inositol polyphosphates are represented on an expanded scale in (b) and show noradrenaline (NA)-stimulated accumulation of IP_2 and a small peak corresponding to IP_3: (○) = basal; (●) = NA (10^{-3} M)-stimulated. The total radioactivity eluted by each formate solution was obtained using separate columns in the same experiment (c). GPI was eluted with 30 ml of 5 mM $Na_2B_4O_7$/60 mM HCOONa, and 20 ml of 0.2, 0.4, and 1.0 M $HCOONH_4$/0.1 M HCOOH was used to elute IP_1, IP_2 and IP_3 respectively. The results shown are representative of four similar experiments: open columns indicate basal radioactivity; cross-hatched columns show NA (10^{-3} M)-stimulated activity.

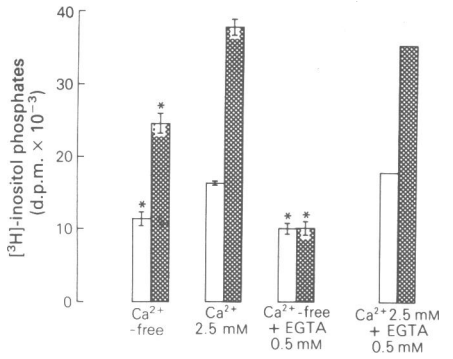

Figure 5 Effects of Ca^{2+} concentration on noradrenaline (NA)-stimulated inositol phospholipid hydrolysis in rat kidney cortex. Slices were incubated in Ca^{2+}-free buffer with [^{3}H]-inositol for 60 min, washed thoroughly and then incubated in either Ca^{2+} (2.5 mM), Ca^{2+}-free, Ca^{2+}-free + EGTA (0.5 mM) or Ca^{2+} (2.5 mM) + EGTA (0.5 mM) with or without NA (10^{-3} M) for 60 min. Data are presented as the mean of three separate experiments conducted in duplicate except for the Ca^{2+} (2.5 mM) + EGTA (0.5 mM) treatment which was a single experiment; vertical lines show s.e.mean. Open columns indicate basal radioactivity, cross-hatched columns show NA stimulated activity.
*Significant differences ($P < 0.01$, Student's t test) from identically treated slices incubated in Ca^{2+} (2.5 mM).

NA > phenylephrine > methoxamine, (+)-NA. EC_{50} values for agonists are presented in Table 2. Neither the α_2-adrenoceptor agonist clonidine nor the β-adrenoceptor agonist (−)-isoprenaline stimulated accumulation of IP's at concentrations up to 100 μM.

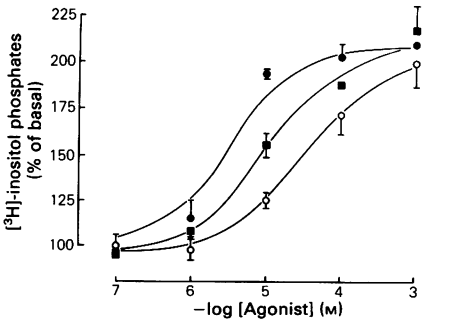

Figure 6 Stimulation of accumulation of [^{3}H]-inositol phosphates by α-adrenoceptor agonists in rat kidney cortex: (●) (−)-noradrenaline; (○) methoxamine; (■) phenylephrine. Labelled slices were incubated with 5 concentrations of agonist for 60 min. Data are means of at least 4 separate experiments conducted in duplicate; vertical lines show s.e.mean.

Thus, the agonists stimulated the production of [^{3}H]-IP's in a manner consistent with it being an α_1-adrenoceptor-related process.

This was confirmed in studies where a range of adrenoceptor antagonists were used which produced parallel rightward shifts of the concentration-response curve to (−)-NA, e.g. prazosin (100 nM) (Figure 7). The relative affinities (pA_2 values) of antagonists were prazosin > BE2254 >> phentolamine >> idazoxan > rauwolscine (Table 2) and show an affinity profile consistent with an interaction at α_1-adrenoceptors.

Autoradiographic and membrane binding studies in rat kidney have indicated that α_1-adrenoceptors are mainly localized to the renal cortex where they are associated with proximal tubules (Summers, 1984). In cortical slices levels of [^{3}H]-IP's (d.p.m.) rose to 180% of control and in medullary slices to 118% of control in response to (−)-NA (100 μM), indicating that the major population of α_1-adrenoceptors linked to PI hydrolysis is in the renal cortex (Figure 8a). Although incorporation of [^{3}H]-inositol into cortical slices was three times higher than that into medullary slices (Figure 8b), the basal rates of accumulation of [^{3}H]-IP's were similar (Figure 8a). An index of turnover rates obtained by expressing the results as fmol [^{3}H]-IP's/[^{3}H]-inositol incorporated, indicates that the pool of inositol phospholipids in the medulla is smaller, but has a faster rate of turnover (Figure 8c).

Effects of indomethacin on basal and NA induced accumulation of IP's in kidney slices

The presence of Ca^{2+}-dependent accumulation of IP's in control 'unstimulated' slices was considered an indication of the presence of endogenous stimulants. In the presence of indomethacin (10 μM) there was no significant change in the basal accumulation of [^{3}H]-IP's (control 48,050 ± 996 d.p.m.; indomethacin 42,626 ± 1,449 d.p.m.). Accumulation in the presence of NA (1 mM) was significantly decreased by indomethacin (control 84,276 ± 3,957 d.p.m.; indomethacin 73,207 ± 3,037 d.p.m., $n = 3$, $P < 0.05$).

Discussion

The mechanism employed by calcium-mobilizing receptors for the transduction of neurotransmitter information is thought to be the turnover of membrane phosphoinositides (Michell, 1975; Downes, 1982; Berridge, 1984). Activation of receptors is followed by the rapid hydrolysis of PIP_2 by phospholipase C, and the production of at least two putative second messengers, DAG and IP_3 which induce an increase in cellular activity (Berridge, 1984; Nishizuka, 1984). A characteristic feature of this mechanism is the multifunctional nature of the res-

Table 2 Effect of α-adrenoceptor agonists and antagonists on accumulation of [^{3}H]-inositol phosphates in rat renal cortical slices

Agonists	*Gluconeogenesis* EC_{50} μM	*[^{3}H]-prazosin binding* K_i μM	*PI hydrolysis* EC_{50} μM
(−)-Noradrenaline	0.10 ± 0.02	9.8 ± 1.2	4.28 ± 1.09
(+)-Noradrenaline	ND	ND	42.5 ± 6.7
Methoxamine	ND	ND	40.3 ± 8.5
Phenylephrine	0.49 ± 0.17	13.0 ± 2.2	13.0 ± 3.0
Antagonists	pK_B	pK_i	pA_2
Prazosin	8.83 ± 0.06	8.81 ± 0.47	8.48 ± 0.07
BE2254	9.13 ± 0.15	9.03 ± 0.01	8.04 ± 0.09
Phentolamine	7.56 ± 0.01	7.26 ± 0.04	7.26 ± 0.09
Idazoxan	ND	ND	5.83 ± 0.07
Rauwolscine	ND	ND	5.25 ± 0.03

IC_{50} values were obtained using computer-assisted curve fitting (McPherson *et al.*, 1983) of the concentration-response relationship. pA_2 values were calculated from shifts to the right of the (−)-NA concentration-response curve with single concentrations of antagonists. Values for gluconeogenesis and [^{3}H]-prazosin binding are taken from McPherson & Summers, (1982). Figures given represent mean ± s.e.mean (n = 3–6). ND = not determined.

ponse (Michell, 1975) in that production of these second messengers leads to a host of secondary effects in the cell (Berridge, 1984). Evidence for multiple responses is seen in kidney, where activation of α_1-adrenoceptors leads to increased sodium reabsorption (Osborn *et al.*, 1983; Hesse & Johns, 1984), prostanoid production (Cooper & Malik, 1985), gluconeogenesis (Kessar & Saggerson, 1980; McPherson & Summers, 1982), renal vasoconstriction (Schmitz *et al.*, 1981; Smyth *et al.*, 1984; Cooper & Malik, 1985) and inhibition of renin release (Matsumura *et al.*, 1985). In the light of the large quantity of physiological data, it is of great interest that the present study demonstrated that renal α_1-adrenoceptors are linked to PI metabolism and that the products of the PI cycle can now be investigated as possible mediators of the diverse array of physiological processes occurring in response to α_1-adrenoceptor activation in the kidney.

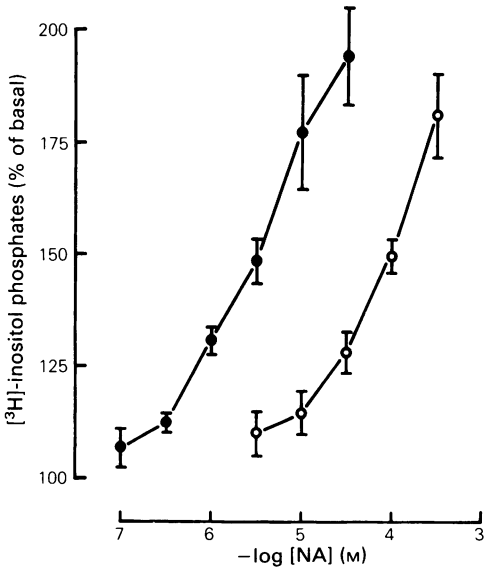

Figure 7 Effects of prazosin on noradrenaline (NA)-stimulated accumulation of [^{3}H]-inositol phosphates in rat kidney cortex. Slices were incubated with prazosin (100 nM) for 20 min before addition of increasing concentrations of NA: (●) control; (○) prazosin. Data are presented as means of 4 separate experiments conducted in duplicate; vertical lines show s.e.mean.

The relationship between PI hydrolysis and Ca^{2+} mechanisms is well documented in other systems (Michell, 1975; Downes, 1982). In effect, a role of PI hydrolysis in receptor-mediated signal transduction relies on establishing that hydrolysis precedes and is responsible for Ca^{2+} movements in the cell (Berridge, 1984). Not only is the mobilization of intracellular Ca^{2+} induced by PI hydrolysis important for increased cellular activity but it is also important in the regulation of precursor phospholipid synthesis (Hayashi & Amakawa, 1985). Labelling of phospholipid pools with [^{3}H]-inositol is dependent upon the Ca^{2+} concentration and since Ca^{2+} inhibits [^{3}H]-inositol incorporation in brain (Kendall & Nahorski, 1984 and the present paper), lowering of Ca^{2+} levels is one strategy adopted to increase PI labelling. This was not required in the present study since concentrations of Ca^{2+} which inhibited incorporation of [^{3}H]-inositol in brain, stimulated incorporation in kidney. The differential effect of Ca^{2+} in the two tissues indicates a complex

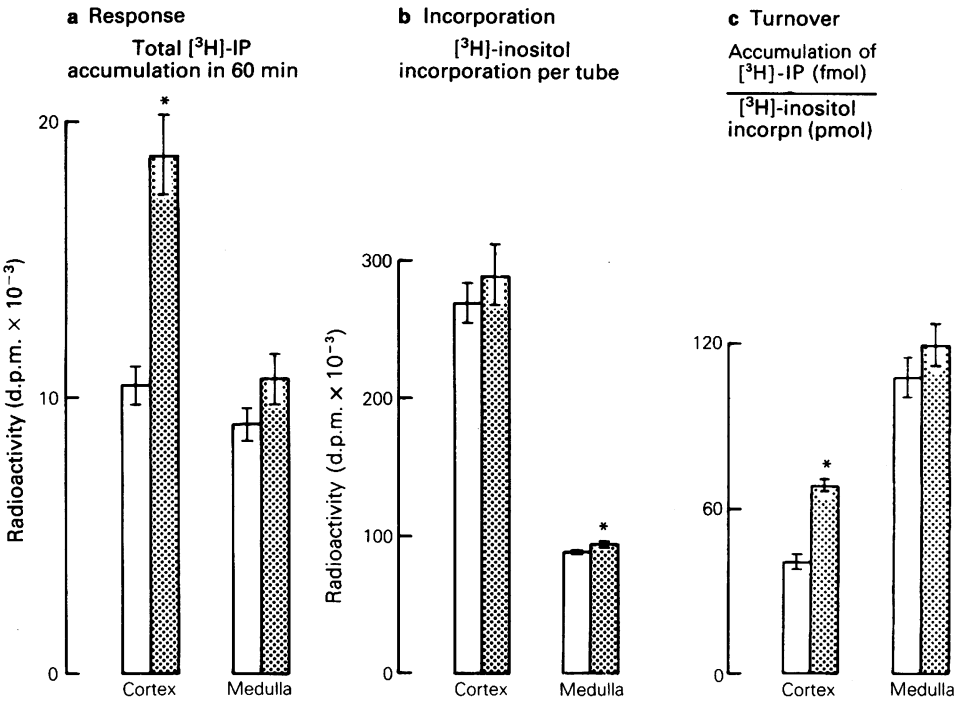

Figure 8 Regional localization of α_1-adrenoceptor-mediated stimulation of [^{3}H]-inositol phospholipid metabolism. Slices prepared from cortex and medulla were labelled with [^{3}H]-inositol and incubated with noradrenaline (NA, 100 μM) for 60 min. Both the accumulation of [^{3}H]-inositol phosphates [^{3}H]-IP (a) and incorporation of [^{3}H]-inositol into phospholipids (b) were measured and compared to that in control unstimulated slices (c) turnover was also measured: open columns, control; stippled columns, incubated with NA. Data are presented as means of three separate experiments conducted in triplicate; vertical lines show s.e.mean. Asterisks indicate where changes produced in stimulated slices significantly differ from controls ($P < 0.05$, Student's t test).

relationship between Ca^{2+} and enzymes in the PI synthesis and phosphorylation steps (Abdel-Latif, 1983).

The effect of Ca^{2+} was studied on basal and NA-stimulated production of [^{3}H]-IP's. Both NA-stimulated and unstimulated levels were increased in the presence of Ca^{2+} (Figure 5). As in brain (Kendall & Nahorski, 1984) the NA response was Ca^{2+}-dependent since it was abolished by EGTA (0.5 mM). These effects of Ca^{2+} could not be due to effects on incorporation since in these experiments, kidney slices were labelled with [^{3}H]-inositol and washed before being exposed to the various treatments. Since the basal production of IP's was also Ca^{2+}-dependent this may indicate the production of endogenous substances that act on their own receptor systems to promote phospholipid hydrolysis. Experiments with indomethacin may suggest the involvement of arachidonic acid metabolite(s) and experiments are in progress to test this hypothesis. Activation of α_1-adrenoceptors in this tissue is associated with the release of prostanoids (Cooper & Malik, 1985) and these, or related substances such as thromboxanes and endoperoxides are likely candidates. The indomethacin-sensitive component of the PI response may be only one component of the Ca^{2+}-stimulated increased control levels of [^{3}H]-IP's. Gluconeogenesis is a calcium-dependent process stimulated by activation of α_1-adrenoceptors in rat kidney (Kessar & Saggerson, 1980; McPherson & Summers, 1982). Glucose is able to cause a PI effect in liver (Fex & Lernmark, 1972) and so may also warrant consideration as a candidate for the role of an endogenous stimulant of PI turnover.

Localization of α_1-adrenoceptors with [^{3}H]-prazosin has shown that binding is to the renal cortex where the receptors are associated with proximal tubules and small blood vessels (Summers, 1984). Here, we have shown that receptors with characteristics similar to those identified in binding studies are coupled to inositol phospholipid breakdown. The response to NA was much larger in the cortex than in medulla supporting the findings of the binding and

autoradiographic studies. In competition studies, pA_2 values for all drugs were in close agreement with those obtained for inhibiting gluconeogenesis in rat kidney (McPherson & Summers, 1982) and for inhibiting NA-induced increases in [^{3}H]-inositol metabolism in rat cerebral cortex (Brown *et al.*, 1984; Minneman & Johnson, 1984). The concentration-response curves for the agonist-stimulated PI hydrolysis lie at least one order of magnitude to the right compared to those obtained for gluconeogenesis (McPherson & Summers, 1982) (Table 2). This observation illustrates that the transducer acts to amplify the response since relatively low levels of hydrolysis will result in near maximal stimulation of gluconeogenesis.

A characteristic feature of the PI response is its multifunctional nature (Michell, 1975; Berridge, 1984). Known biochemical consequences of PI hydrolysis include mobilization of Ca^{2+}, activation of protein kinase C, stimulation of guanylate cyclase and release of arachidonic acid (for review, see Berridge, 1984). Several responses of the cell may ensue such as the release of prostanoids in rat kidney by α-adrenoceptor agonists (Cooper & Malik, 1985). This effect is partially dependent upon intracellular Ca^{2+} suggesting a possible role for IP_3 the intracellular mediator of Ca^{2+} mobilization (Streb *et al.*, 1983). The other product of PI hydrolysis, DAG, is a potential source of arachidonic acid (Bell *et al.*, 1979) and hence prostanoids. Alternatively, arachidonic acid may be generated from other membrane phospholipids by Ca^{2+} stimulation of phospholipase A_2 (Billah *et al.*, 1980). Another response, gluconeogenesis, is stimulated by activation of α_1-adrenoceptors in rat kidney and is partially Ca^{2+}-dependent (Kessar & Saggerson, 1980; McPherson & Summers, 1982). Hormonal regulation of gluconeogenesis is thought to occur at specific points (Kraus-Friedmann, 1984). For substrates entering as pyruvate, rate-limiting steps involving protein phosphorylation via the Ca^{2+}-binding protein calmodulin occur between the conversion of mitochondrial pyruvate to cytosolic phosphoenolpyruvate. By activating calmodulin, increased levels of Ca^{2+} may promote the conversion of lactate and pyruvate into glucose production. Alternatively, protein phosphorylation by protein kinase C may be important since this enzyme is activated by DAG (Nishizuka, 1984). The consequences of PI hydrolysis may also affect Na^+ reabsorption which is stimulated by α_1-adrenoceptor activation in kidney. Most Na^+ is reabsorbed with Cl^- but some is reabsorbed by an active process by which Na^+ enters in exchange for one proton (Ganong, 1979). Na^+ reabsorption may be enhanced by a similar mechanism to that whereby phorbol esters increase intracellular pH in Swiss mouse 3T3 cells (Burns & Rozengurt, 1983). DAG may stimulate protein kinase C to activate a Na^+/H^+ carrier (Berridge, 1984) although this system in rat kidney requires investigation.

In conclusion, we have demonstrated α_1-adrenoceptors in rat kidney linked to PI hydrolysis using a direct measure of the products of phospholipid breakdown, the inositol phosphates. It is proposed that the many physiological responses which occur following activation of renal α_1-adrenoceptors, including gluconeogenesis, sodium reabsorption and prostanoid release are direct consequences of the cascade of events which occur following inositol phospholipid breakdown. It is suggested that the α_1-adrenoceptor in rat kidney provides a suitable model system for the study of the relationships between receptor activation, PI hydrolysis and the production of end/organ responses.

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