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THE POSSIBLE INVOLVEMENT OF CHANGES IN PHOSPHOINOSITOL TURNOVER IN THE RESPONSES OF RENAL SODIUM TRANSPORT TO NORADRENALINE

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Abstract—Noradrenaline stimulated the formation of [^{3}H]inositol monophosphates from ^{3}H-inositol-phospholipids in rat kidney cortex slices. This response was inhibited by the α_1-adrenoceptor antagonist prazosin but not by the α_2-adrenoceptor antagonist yohimbine, suggesting that the response was mediated via an α_1 adrenoceptor. This is in keeping with the ability of noradrenaline to increase sodium transport in this tissue by α_1-adrenoceptor stimulation.

A contribution to the regulation of renal tubular sodium transport by the sympathetic nervous system has been well established. Changes in renal sympathetic nerve activity, produced either directly [1, 2] or by modification of physiological reflexes [3, 4], induce changes in renal sodium and water reabsorption which are mediated directly by adrenergic nerve terminals in contact with renal tubular epithelial cells. The proximal tubule has been identified as one site of action for this neural control mechanism for solute and water transport [5] and current evidence supports the view that the mechanism operates via tubular α-adrenoceptors [6]. Radioligand binding and autoradiographic studies have identified both α_1- and α_2-adrenoceptor binding sites in the cortex of the rat kidney [7, 8] and both receptor subtypes appear to be located post-junctionally since pretreatment with 6-hydroxydopamine does not reduce binding at either receptor [9]. Further it was recently demonstrated [10] that the enhancement of sodium transport by noradrenaline by rat kidney cortex slices appears to be mediated by α_1-adrenoceptors.

The post-receptor cellular mechanisms involved in the renal tubular responses to noradrenaline are not understood, but in the rat kidney slice preparation, a rise in intracellular calcium due to an influx of extracellular calcium and also a change in ribosomal protein synthesis appear to be involved [10].

Many α_1-adrenoceptor-mediated events have been shown to involve change in phosphoinositide (PI) turnover [11]. The following experiments were carried out to measure changes in PI turnover in rat kidney cortex slices induced by noradrenaline and α_1 and α_2 directed adrenoceptor agonists. A comparison was also made between the effects of α_1 and α_2 directed adrenoceptor antagonists on noradrenaline-stimulated PI turnover and on sodium transport. The results obtained are consistent with the view that an increase in PI turnover is involved, at least in part, in the stimulation of renal sodium transport following the application of noradrenaline.

MATERIALS AND METHODS

Phosphoinositide turnover. The method was a modification of that described by Berridge *et al.* [12]. Male Wistar rats (250 g) were killed by cervical dislocation, the kidneys removed and placed in freshly oxygenated bicarbonate buffer. The composition of the buffer was 123 mM NaCl, 5 mM KCl, 0.8 mM $CaCl_2$, 1.3 mM $MgCl_2$, 1.4 mM KH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose and 10 mM LiCl. The cortex of the kidneys were sliced with a McIlwain tissue chopper into 150 μM cubes. The resulting fragments were then incubated in conical flasks at 37° for 30 min with three changes of gassed incubation medium. This was followed by an incubation in the presence of 10 μCi [^{3}H]-myoinositol/ml at 37° for 180 min during which the flasks were gassed with 5% CO_2 in O_2 at 20 min intervals. This incubation was terminated by washing the slices in ice cold, isotope-free incubation buffer.

Aliquots (25 μl) of packed tissue (2.01–2.40 mg/protein) were then dispensed into vials containing incubation buffer to a final volume of 300 μl. The vials were gassed with 5% CO_2/95% O_2 and incubated for a further 5 min during which, in most cases, an α-adrenoceptor antagonist was added. The reaction was initiated by the addition of an α-adrenoceptor agonist or vehicle and the tubes re-gassed and incubated for a further 45 min.

The incubations were terminated by the addition of 1 ml chloroform/methanol (2:1 v/v). The phases were then separated by the addition of chloroform (0.31 ml) and distilled water (0.31 ml). Inositol phosphates were separated by chromatography of the aqueous layer over Dowex formate anion exchange columns.

Transport studies. The rat kidney cortex slice technique is described in detail by Poat and Munday [13]. Slices of rat renal cortex were preloaded with sodium and depleted of potassium by a 10 min incubation at 37° under N_2 in 30 ml of buffer (composition 143 mM

NaCl, 3 mM $CaCl_2$, 1.5 mM $MgSO_4$ and 3.5 mM sodium phosphate buffer pH 7.4). Following this incubation, slices were transferred to 25 ml conical flasks (5–7 mg dry wt slice per flask) and incubated in the presence or absence of drug for 8 min at 22°. Pump activity was initiated by the addition of oxygen and glucose (10 mM). At the end of both incubation periods slices were rinsed, blotted, dried and weighed. The slices were ashed with nitric acid and the residue dissolved in hot, deionised water and assayed for sodium and potassium content by flame photometry.

Sodium transport was calculated as the difference in sodium content of the slices before and after the second incubation. Sodium transport is expressed in terms of μmol/g dry wt extruded during the second incubation. Results are expressed as a mean ± SEM and statistical significance of differences between means was determined by Student's *t*-test.

RESULTS

In rat renal cortex slices adrenaline and noradrenaline stimulated the formation of [^{3}H]-inositol monophosphates in the presence of LiCl (10 mM), with EC_{50}s of 0.86 ± 0.31 and 1.12 ± 0.22 μM respectively (N = 4). Furthermore the α_1-adrenoceptor agonist phenylephrine also stimulated the accumulation of [^{3}H]-inositol monophosphates with an EC_{50} of 4.16 ± 1.21 μM (Fig. 1). Clonidine, isoprenaline and dopamine were without effect at concentrations up to 100 μM. This suggests that the increase in the accumulation of the labelled inositol phosphate was due to a stimulation of α_1-adrenoceptors, as suggested in other tissues [11, 14]. This assumption was confirmed by experiments in which slices were incubated with noradrenaline in the presence of a variety of α-adrenoceptor antagonists. The

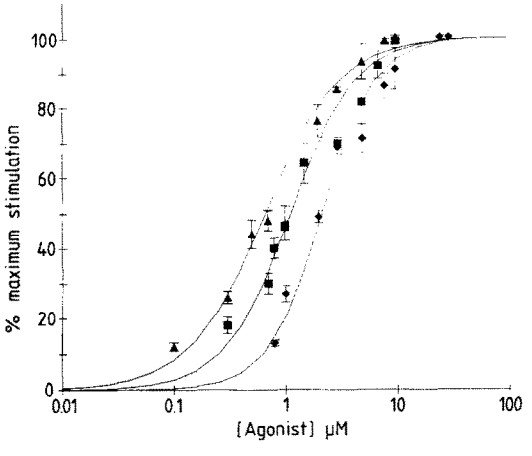

Fig. 1. Stimulation of accumulation of [^{3}H]-inositol monophosphate in rat kidney cortex slices by α-adrenoceptor agonists. Slices were preincubated with [^{3}H]-inositol and drugs (▲, adrenaline; ■, noradrenaline; and ◆, phenylephrine) added and incubated for 45 min at 37°. The reaction was terminated and labelled inositol phosphates separated as described in Materials and Methods. Basal incorporation was 600 dpm/mg tissue and stimulation expressed as % maximum response where maximum response with 10 μM noradrenaline represented a 6–8-fold stimulation.

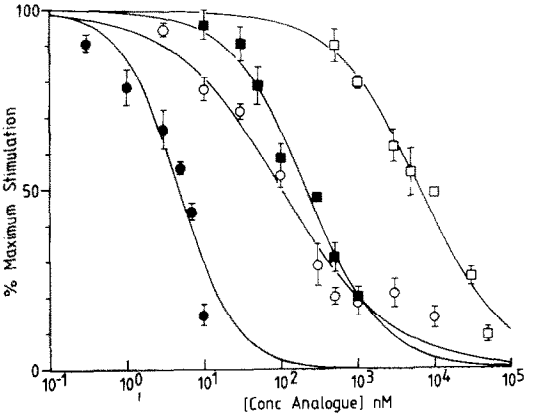

Fig. 2. Inhibition of noradrenaline stimulated accumulation of [^{3}H]-myo-inositol-monophosphate in rat kidney cortex slices. Drugs (●, prazosin; ■, phentolamine; □, yohimbine; and ○, indoramine) were added 5 min before the reaction was started by the addition of 5 μM noradrenaline, and terminated after 45 min. Noradrenaline stimulated the basal incorporation from 1052 dpm/mg tissue to 8540 dpm/mg tissue in the absence of drug.

results in Fig. 2 show that the order of potency for inhibition of the labelling was prazosin (IC_{50} 4.43 ± 0.32 nM) > indoramine (IC_{50} 126.00 ± 26.00) > phentolamine (IC_{50} 218.30 ± 3.20) > yohimbine (IC_{50} 6980.00 ± 125.00) (N = 3).

Although a functional role for α_1-adrenoceptors in the kidney is not yet clear, there is evidence that stimulation of this receptor might be involved in sodium transport. Accordingly we examined the effects of adrenoceptor agonists and antagonists on sodium transport using a rat renal cortical slice preparation [13]. In this simple preparation the slices are preloaded with sodium by an anaerobic incubation in the absence of glucose and subsequently sodium pump activity initiated by the addition of an energy source and oxygen. Since [16] noradrenaline has been shown previously to stimulate sodium extrusion by a mechanism independent of potassium in the external medium, potassium was omitted from both incubations. Noradrenaline, at concentrations greater than 10 μM, stimulated sodium extrusion from preloaded rat renal cortical slices, with a maximum response at 10^{-4} M. The effective concentration could be reduced by the inhibition of noradrenaline uptake and the inhibition of its metabolism (see Table 1). Addition of pargyline and nomifensine to slices had no effect on the EC_{50} of noradrenaline in stimulating phosphatidyl inositol metabolism. The ability of noradrenaline to stimulate active sodium extrusion was inhibited by α-adrenoceptor antagonists with the order of potency = prazosin (IC_{50} 5.00 ± 0.70 nM) > indoramine (IC_{50} 72.00 ± 32.00 nM) > yohimbine (IC_{50} 3 ± 0.193 μM). Again this order of potency suggests that this functional response to noradrenaline is due to the stimulation of α_1-adrenoceptors.

The effects of adrenoceptor antagonists on PI turnover and sodium transport in renal slices and on the binding of the α_1-adrenoceptor antagonist

Table 1. The effect of noradrenaline on sodium transport by sodium loaded rat renal cortex slices incubated at 22° for 8 min in the presence of glucose and the absence of potassium

Noradrenaline conc.	Sodium loss (μmol/g dry wt)	Significance from control
Control	96 ± 14 (8)	
10^{-5} M	84 ± 17 (6)	NS
10^{-4} M	198 ± 14 (8)	$P < 0.02$
10^{-3} M	180 ± 18 (6)	$P < 0.02$
+pargyline (5×10^{-5} M) and nomifensine (1×10^{-5} M)		
Control	103 ± 16 (6)	
10^{-5} M	165 ± 14 (6)	$P < 0.02$

[^{3}H]prazosin are shown in Table 2 expressed as IC_{50} values. The table shows a good agreement in the values for antagonist effects on the three responses inferring a relationship. For the agonists such as noradrenaline the concentrations needed for the sodium transport response were one order of magnitude greater than those needed to stimulate PI turnover or inhibit specific [^{3}H]prazosin binding.

Thus it is possible that noradrenaline stimulates α_1-adrenoceptors leading to an increase in the phosphatidyl-inositol pathway as a secondary messenger response leading to an increase in sodium transport in the rat renal cortex.

DISCUSSION

The results presented demonstrate that noradrenaline stimulates both sodium transport and phosphatidyl inositol metabolism in kidney cortex slices.

It is now accepted that in the presence of agonist phosphatidyl inositol 1,4-bisphosphate is rapidly hydrolysed forming two potential second messengers DAG and IP_3 [14]. The continuous production of intermediates can be prevented by LiCl which inhibits the enzyme inositol monophosphatase leading to a build up of inositol monophosphate in the presence of agonists stimulating the pathway. Thus, as in the present situation, increased PI turnover can be assessed by the incorporation of tritium from [^{3}H]-inositol into (^{3}H)-inositol-l-phosphate in the presence of LiCl [14]. Noradrenaline has been reported to stimulate PI turnover in rat brain hippocampus [17] and cortex [18] and in rat parotid [19] using this method. The results presented extend these observations to the rat kidney where noradrenaline was 10-fold more potent in stimulating PI turnover than in other preparations. This probably can be explained from the pre-labelling regime used in the present experiments resulting in increased sensitivity of the preparation as this method ensures extremely low blank values. Alternatively a difference in potency could well be explained by differences in receptor reserve in the tissues under study.

Production of these two second messenger systems can lead to many secondary effects [14] and this diversity of response is in keeping with the system being responsible for the many renal functions ascribed to α-adrenoceptor stimulation. Thus α_1-adrenoceptors are implicated in sodium transport, gluconeogenesis, renin release and renal haemodynamics. The present study suggests that the sodium transport response is mediated through the α_1-adrenoceptor subtype in that phenylephrine was as potent as noradrenaline, while the α_2-adrenoceptor agonists, clonidine and oxymetazoline, were inactive. Further the response was inhibited by the α_1-adrenoceptor antagonist prazosin with the α_2-adrenoceptor antagonists, rauwolscine and yohimbine, being weak inhibitors. The potency of prazosin in this preparation compares well with the pA_2 values of 8.29 and 9.82 reported for studies on brain slices [14, 18]. During the preparation of this paper Neylan and Summers [20] produced evidence for the role of PI turnover in renal gluconeogenesis, a response thought to be mediated through α_1-adrenoceptor stimulation.

The ability of noradrenaline to affect renal transport is well documented and is shown here using kidney cortex slice preparation. The relative potencies of a range of selective α-adrenoceptor antagonists demonstrated that both sodium extrusion from slices and PI turnover were highly sensitive to α_1-adrenoceptor antagonists. The order of potency of a range of antagonists on these two responses was in agreement with the potency profile of these antagonists in displacing [^{3}H]-prazosin binding to renal cortical membranes again suggesting that an α_1-adrenoceptor is involved in both PI and sodium transport. In contrast noradrenaline was an order of magnitude less potent on sodium transport when compared with PI hydrolysis or [^{3}H]-prazosin binding, although the "all or none" nature of the

Table 2. The effect of adrenoceptor antagonists on [^{3}H]-prazosin binding, tritium incorporation into [^{3}H]-inositol-monophosphate and sodium transport in rat renal tissue

	PI response IC_{50} (nM)	* [^{3}H]prazosin binding IC_{50} (nM)	Sodium transport IC_{50} (nM)
Prazosin	4.4	3.2	5.0
WB 4101	NT	119.0	100.0
Indoramine	126.0	126.0	72.00
Yohimbine	6980.0	2400.0	3000.0
Propranolol	Inactive	>10000	Inactive
(±)Noradrenaline	1120.0	3400.0	≃50000.0

* Results taken from Cotterell *et al.* [15]; NT = Not tested.

transport response precludes meaningful agonist dose–response curves. The addition of pargyline and nomifensine was able to reduce the effective concentration of noradrenaline to a similar value for the stimulation of both PI hydrolysis and sodium transport. The inhibitors, however, only effected the transport response and their lack of effectiveness on noradrenaline stimulation of PI hydrolysis is difficult to explain.

The similar relative potencies of α-adrenoceptor antagonists on noradrenaline-stimulated sodium transport and PI turnover indicates the possibility that an increase in PI turnover may be involved in the transduction processes between receptor occupation by noradrenaline and the response, namely, the enhancement of sodium transport. The exact sequence of events from binding at the receptor through stimulation of the secondary messenger to transport response are unclear although calcium mobilisation would appear to be intimately involved [10]. Many of the known consequences of stimulated PI hydrolysis are calcium-dependent/calcium activating events [14]. It may be relevant that one of the second messengers, produced as a result of PI hydrolysis, diacylglycerol, has been implicated in the activation of a Na/H carrier [14] and this second messenger may have a wider role in the control of renal transport processes.

The results presented demonstrate an increase in PI turnover in rat kidney cortex slices which has similar sensitivity to noradrenaline and susceptibility to α-adrenoceptor antagonists as does the sodium transport response. Such findings are consistent with the view that phosphatidyl inositide intermediates play a role, at least in part, in the modulation of renal sodium transport by noradrenaline.

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