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Abnormal vascular phosphoinositide hydrolysis in the spontaneously hypertensive rat

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1 The production of [^{3}H]-inositol phosphates was studied in labelled segments of aorta from spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) controls at 5 and 19 weeks, either unstimulated or in the presence of noradrenaline.

2 Basal hydrolysis of inositol phospholipids was significantly enhanced in young SHR ($P < 0.05$) compared to controls but this difference was no longer detected at 19 weeks.

3 Noradrenaline increased [^{3}H]-inositol phosphate accumulation in both SHR and WKY, but maximal hydrolysis was significantly greater in WKY ($P < 0.01$), although the ED_{50} was similar in both groups of animals.

4 These data demonstrate that phosphatidylinositide hydrolysis is enhanced in the young hypertensive rat at the time blood pressure is rising, but that this activity has declined by the time hypertension has reached an established phase. In addition, α_1-agonist induction of inositol phospholipid hydrolysis differs in the two species of animals, being reduced in genetically mature hypertensive rats.

Introduction

Essential hypertension is a disease characterized by an increase in peripheral vascular resistance (Haeusler & Haefely, 1970). Two factors appear to be implicated in this phenomenon: a hypersensitivity of blood vessels to vasoconstrictor stimuli, and structural changes in blood vessel walls notably media thickening. The responses to neurotransmitters have been extensively studied but the overall picture is unclear. Results of experiments using intact animals or vascular beds suggest that noradrenaline sensitivity is greater in spontaneously hypertensive rats (SHR) than in Wistar Kyoto (WKY) controls (Lais *et al.*, 1974; Berecek *et al.*, 1980). Experiments using isolated preparations suggest that it is only part of the SH vasculature which shows increased sensitivity. Femoral small arteries in SHR do not have increased noradrenaline sensitivity (Mulvany *et al.*, 1982), whereas mesenteric vessels do (Mulvany *et al.*, 1980), provided that the increased uptake of noradrenaline by nerve terminals in the vessels of SHR is inhibited. These differences in sensitivity to noradrenaline suggest either that there are differences in various receptors or there are differences in the manner in which the final trigger for contraction is controlled. The affinity of α-receptors for noradrenaline is normal in SHR (Strecker *et al.*, 1975). Thus, differences in the processes which couple receptor-agonist interactions with contraction are likely. In this regard, there is increasing evidence that inositol phospholipids play a role in signal transduction (Berridge & Irvine, 1984). Activation of α_1-adrenoceptors causes hydrolysis of polyphosphoinositides in the cell membrane particularly phosphatidylinositol-4,5 bisphosphate (Campbell *et al.*, 1985). This compound is hydrolysed to inositol-1,4,5 trisphosphate and diacylglycerol, and it has been suggested that this may be the primary event in signal transduction for calcium mobilizing receptors. Inositol-1,4,5 trisphosphate has been shown to mobilize calcium from intracellular stores when added to permeabilized cells (Streb *et al.*, 1983). Because of the increasing evidence that activation of inositol phospholipid metabolism may be involved in signal transduction mediated by α-receptor activation it was decided to investigate this phenomenon in vascular tissue from SHR and WKY in order to examine whether this pathway is abnormal in animals genetically at risk of hypertension.

Methods

Animals

Male SH rats ages 5 and 19 weeks were used and compared to matched male WKY control animals.

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Blood pressures were measured using tail plethysmography.

Tissue preparation and [^{3}H]-inositol phosphate accumulation

The method employed to study phosphate accumulation was modified from that described by Berridge *et al.* (1982).

On the day of study animals were killed by cervical dislocation and the thoracic aorta dissected free and placed in tissue culture medium (M199) containing (mM): NaCl 137, KCl 5.4, $MgSO_47H_2O$ 0.81, $Na_2HPO_47H_2O$ 0.36, $CaCl_2$ 1.26, $NaHCO_3$ 4.2, KH_2PO_4 0.44, $MgSO_47H_2O$ 0.81, $Fe(NO_3)9H_2O$ 0.02. Each aorta was dissected free of fat and divided into segments approximately 4 mm in length. To each segment was added noradrenaline in doses ranging from 0 to 10^{-4} M and 10^{-6} M [^{3}H]-myoinositol. The aortic segments were then incubated at 37°C for 120 min in 200 µl M199 containing LiCl 10^{-2} M, BSA 4 mg ml^{-1} and ascorbate 20 µg ml^{-1}. In this manner in 19 week animals a dose-response curve could be constructed from each thoracic aorta obtained. However, 5 week old rats were too small to allow a full dose range to be tested. Therefore, it was decided to study phosphoinositide hydrolysis in these animals in the presence of noradrenaline at 0 and 10^{-4} M only. All experiments on SH animals were performed at the same time as a control animal was studied. After incubation, the vessels were blotted on filter paper and weighed before being homogenized in 0.5 ml chloroform:methanol: HCl (20:40:1 v/v) at 4°C. The homogenate was left on ice for 10 min after which time 0.5 ml chloroform was added followed by 0.5 ml water. The tubes were agitated and centrifuged at 2000 *g* to separate the aqueous and organic phases, and the aqueous layer was neutralized and added to a column containing 0.5 ml of Bio-Rad AG 1-x8 anion exchange resin in the formate form. The resin was washed 5 times with 5 mM myoinositol and subsequently washed with 0.1 M formic acid/1 M ammonium formate solution to elute bound tritiated inositol phosphate. Two ml aliquots of the eluate were added to 12 ml OptiPhase 'T' scintillant and radioactivity was counted using a Packard Scintillation counter. The amount of [^{3}H]-inositol phosphate accumulated was expressed as counts per min (c.p.m.) per mg of aorta to correct for structural differences in blood vessels from SHR compared to WKY.

Results are expressed as mean ± s.e.mean and agonist-induced responses expressed as percentage of basal c.p.m. per mg of aorta. Results between SH and WKY groups were compared by means of Student's unpaired *t* test in 5 week animals. In the 19 week old animals the dose-response curves were compared by means of two way analysis of variance.

Drugs and isotopes

Noradrenaline hydrochloride, myoinositol and L-ascorbic acid were purchased from Sigma Chemical Company, Poole, Dorset. Bovine serum albumin was obtained from Miles Scientific, Naperville, Illinois, U.S.A., and lithium chloride from Fisons, Loughborough, Leics. [^{3}H]-myoinositol (specific activity 16.5 Ci mmol^{-1}) was purchased from New England Nuclear, Boston, U.S.A. OptiPhase 'T' scintillation fluid was purchased from LKB Instruments, South Croyden, Surrey. Anion-exchange resin AG1-x8 (in the formate form) from Bio-Rad laboratories, Richmond, California, U.S.A. and tissue culture medium M199 from Gibco, Paisley, Scotland.

Results

The mean blood pressure was significantly higher in SHR ($n = 10$) compared to WKY ($n = 10$) at 5 and 19 weeks (5 weeks: 123 ± 4.1 vs 110 ± 3.2 mmHg, $P < 0.03$; 19 weeks: 167 ± 3.6 vs 121 ± 4.7 mmHg, $P < 0.001$). However, the weights did not differ significantly between the two groups at either time (SHR vs WKY: 5 weeks: 99 ± 4.6 vs 90 ± 1.9 g, NS; 19 weeks: 357 ± 6.1 vs 352 ± 5.8 g, NS).

[^{3}H]-inositol metabolism

Five week old rats Unstimulated [^{3}H]-inositol phosphate accumulation was significantly higher in SHR compared to WKY control animals ($P < 0.05$, Table 1). Incubation with noradrenaline (10^{-4} M) resulted in increased inositol phosphate accumulation in both groups and this was significantly higher in SHR ($P < 0.02$, Table 1).

Nineteen week old animals Basal [^{3}H]-inositol phosphate accumulation was now no longer significantly different in the two groups (Table 1), but there was a dose-dependent stimulation of [^{3}H]-inositol phosphate accumulation with noradrenaline in both groups of animals and this was significantly reduced in SHR compared to WKY ($P < 0.03$, Figure 1), although the ED_{50} was different in the two strains (SHR vs WKY: $2.02 \pm 0.5 \times 10^{-6}$ vs $3.0 \pm 1.1 \times 10^{-6}$ M, NS, $n = 10$ in each group).

Discussion

These results demonstrate that phosphoinositide hydrolysis is enhanced in young SHR but that this phenomenon disappears as the animals age. Whilst α-adrenoceptor stimulation is known to promote [^{3}H]-inositol metabolism, this is the first time differences in

Table 1 [^{3}H]-inositol phosphate accumulation in 5 week old and 19 week old SHR and WKY in the absence (unstimulated, basal) and presence of 10^{-4} M noradrenaline

	[^{3}H]-inositol phosphate accumulation (c.p.m. mg^{-1} tissue)					
	5 weeks			*19 weeks*		
	SHR		*WKY*	*SHR*		*WKY*
Basal	656 ± 92 ($n = 10$)	($P < 0.05$)	430 ± 47 ($n = 10$)	887 ± 98 ($n = 10$)	(NS)	798 ± 130 ($n = 10$)
Noradenaline 10^{-4} M	2172 ± 92 ($n = 10$)	($P < 0.02$)	1695 ± 329 ($n = 10$)	2399 ± 129 ($n = 8$)	($P < 0.03$)	3726 ± 542 ($n = 7$)

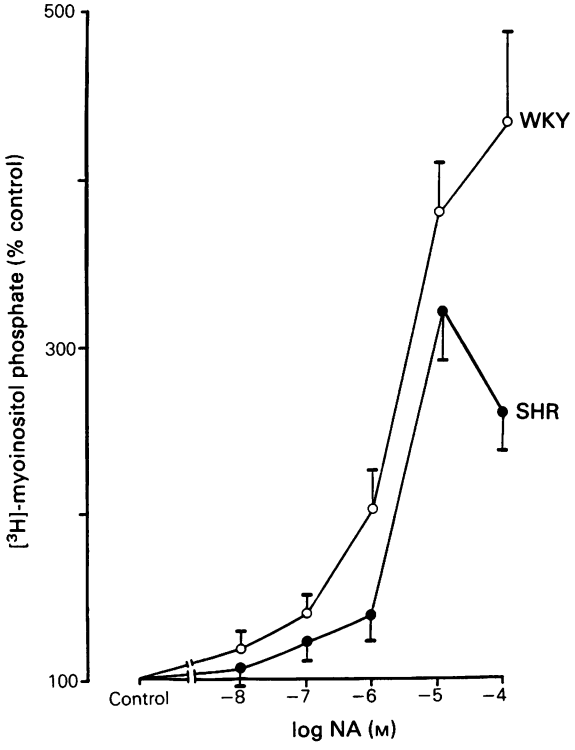

Figure 1 [^{3}H]-myoinositol phosphate accumulation (% control) in 19 week old Wistar Kyoto (WKY) control animals (○) and spontaneously hypertensive rats (SHR) (●) in the presence of increased concentrations of noradrenaline. Each point represents the mean and vertical lines show s.e.mean. $n = 10$ animals in each group.

the agonist-evoked response in vascular tissue from animals genetically prone to hypertension has been studied. Previous experiments studied the kinetics of ^{32}P incorporation in erythrocyte inositol phospholipids in SHR and Wistar rats (Kiselev *et al.*, 1981). Unstimulated ^{32}P incorporation was lower in SHR at 4 weeks but the differences had disappeared at 16 weeks. Conversely a more recent study suggested reduced ^{32}P incorporation into erythrocyte ghost phosphoinositides at 3 and 15 weeks (Koutouzov *et al.*, 1983). However, the same authors have subsequently observed increased erythrocyte phosphoinositide ^{32}P incorporation in Sabra hypertension prone animals compared to Sabra hypertension resistant rats when both groups were fed a low salt diet, high salt diet or treated with DOCA. Studies in vascular tissue have been concerned principally with the demonstration that agonist-receptor interactions stimulate polyphosphoinositide hydrolysis and increase intracellular free calcium levels. In this regard it has been shown that angiotensin II can stimulate hydrolysis and raise calcium concentrations in cultured smooth muscle cells (Wayne Alexander *et al.*, 1985; Nabika *et al.*, 1985; Capponi *et al.*, 1985) and aortic strips (Schoepp & Rutledge, 1984). Because of the importance of α_1-adrenoceptors in controlling the contractile state of smooth muscle, more recent attention has been directed at measuring the stimulation of phosphatidylinositol metabolism by these receptors. In both vas deferens and caudal artery from the rat it has been shown that both noradrenaline and adrenaline caused large, easily quantifiable increases in [^{3}H]-inositol phosphate accumulation (Fox *et al.*, 1985).

The current study confirms noradrenaline mediated inositol phosphate accumulation in vascular tissue and indicates differences in inositide hydrolysis in the SHR. In the young animals when blood pressure was rising but not yet fully established [^{3}H]-inositol phosphate accumulation was enhanced either unstimulated or in the presence of noradrenaline. However, by 19 weeks when the hypertensive process was fully established unstimulated inositol phosphate accumulation was similar in the two groups of animals and noradrenergic evoked responses were diminished in SHR, although the ED_{50} was not different in the two strains indicating no differences in sensitivity to the

agonist. Differences between the two strains of animal are unlikely to be explained in terms of smooth muscle hypertrophy in SHR because the results were corrected on a tissue weight basis. However, this explanation cannot be completely excluded as hypertrophy could be associated with increased connective tissue or tissue water.

Previous work has examined the receptor reserve for α_1-adrenoceptor accumulation of [^{3}H]-inositol phosphates (Minneman & Abel, 1984; Lynch *et al.*, 1985). The dose-response curve for vascular activation of inositide hydrolysis described here is similar to that previously observed (Fox *et al.*, 1985), with maximum hydrolysis seen at concentrations of noradrenaline far above that needed to elicit maximal smooth muscle contraction. It would appear that noradrenaline is more powerful in activating inositide breakdown than activating contraction, indicating a receptor reserve for α_1-receptor-evoked inositide hydrolysis. The difference in inositide metabolism in SHR is potentially extremely important. At the time the blood pressure was rising in SH animals inositol phosphate accumulation was enhanced. Much interest has recently focussed on the role of phosphoinositides in excitation-contraction coupling. Agonist stimulation of vascular tissue produces an increase in [^{3}H]-inositol phosphate accumulation in seconds and this is associated with a rise in intracellular free calcium concentration (Wayne Alexander *et al.*, 1985). In addition, inositol-1,4,5 trisphosphate when applied to permeabilized cells can initiate release of calcium stores and contraction in smooth muscle cells (Somlyo *et al.*, 1985). In the young SHR, therefore, an increased basal turnover of phosphoinositide lipids and a greater agonist-induced response would be consistent with this cellular process being intimately involved in events initiating the blood pressure rise. The sympathetic nervous system is often implicated in the early phase of hypertension. Certainly at 4 weeks there is evidence of increased sympathetic activity (Yamori, 1976) and therefore in vascular tissue enhanced phosphoinositide breakdown would be expected. This would have two consequences: firstly increased tone in vascular tissue in SHR, which has been demonstrated (Hermsmeyer, 1976), and secondly an increased trophic stimulus on the smooth muscle cells. In this context noradrenergic activity has been shown to be a potent growth promoting factor in cultured cells when the pressure load had been removed (Bevan, 1984) and diacylglycerol generation from phosphatidylinositol metabolism has been postulated as being instrumental in providing the stimulus for cell division (Berridge, 1985). Our findings in SHR at 19 weeks suggest that the initial stimulus to raised blood pressure was no longer present and, in fact, not required; for by then medial thickening would be sufficient to maintain the hypertensive process.

This study therefore suggests that a genetic defect in the cell membranes of hypertension-prone animals leads to disturbances of physico-chemical function. In the early phase of hypertension deranged phosphoinositide breakdown may well contribute to increased tone and medial thickening. Further studies must now follow to induce and reverse hypertension in animals whilst phospholipid metabolism is followed.

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