Noradrenaline-stimulated inositol phosphate accumulation in arteries from spontaneously-hypertensive rats

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1 The effects of noradrenaline upon polyphosphoinositide (PPI) breakdown was investigated by measuring the accumulation of inositol phosphates (IPs) in tail arteries from normo- (WKY) and spontaneously-hypertensive (SHR) rats.

2 Noradrenaline (10^{-7} – 10^{-3} M) evoked a concentration-dependent increase in total IP accumulation in both WKY and SHR rats but no significant differences between the populations were detected.

3 In contrast, significant differences in the accumulation of the individual IPs, which contributed to the total IP, occurred. A significantly greater noradrenaline-stimulated accumulation of inositol trisphosphate (IP_3) was observed in tissues from SHR compared with those from WKY rats at each effective concentration of noradrenaline. This was paralleled by an equivalent reduction in inositol monophosphate (IP_1) accumulation, consistent with the lack of a significant difference in noradrenaline-stimulated total IP accumulation between the two populations.

4 In time course studies, an enhanced noradrenaline-induced accumulation of IP_3 in SHR compared to WKY rats, occurred from the earliest time point studied after the addition of the catecholamine both in the presence and absence of LiCl (10 mM). In the presence of LiCl (10 mM) no significant difference in noradrenaline-evoked total IP accumulation between SHR and WKY rats was observed; in the absence of LiCl noradrenaline-evoked a greater total IP accumulation in SHR than in WKY rats at all time points investigated.

5 These studies suggest that the main reason for the enhanced noradrenaline-induced accumulation of IP_3 in arteries from SHR rats is a reduced rate of dephosphorylation of both IP_3 and inositol biphosphate (IP_2) rather than a greater formation of IP_3 from PPIs.

6 This enhanced accumulation of IP_3 may result in an increased calcium mobilisation accounting for the increased contractility to noradrenaline of tail arteries from SHR as compared with those from WKY rats.

Keywords: Hypertension; smooth muscle; vascular; polyphosphoinositides; noradrenaline

Introduction

The enhanced contractile response of the vasculature to both nerve stimulation (Ekas & Lokhandwala, 1981; Muir & Wardle, 1989) and to exogenous catecholamines, such as noradrenaline (Fouda *et al.*, 1987; Muir & Wardle, 1989), in hypertensive compared to normotensive rat models, implicates a postsynaptic locus for this effect. Alterations in signal-transduction systems, recently implicated in other cardiovascular diseases such as hypertension and congestive heart disease (Brodde *et al.*, 1989; Brodde & Michel, 1989), could underlie the development of hypertension and account for the increased contractility of vascular smooth muscle in this disease.

A signal-transduction mechanism closely associated with contraction in vascular smooth muscle, including the rat tail artery (Bao *et al.*, 1989; Schwarze *et al.*, 1990) involves stimulation of polyphosphoinositide (PPI) metabolism to generate inositol phosphates (IPs) and diacylglycerol (DAG) (Abdel-Latif, 1986). Noradrenaline increases intracellular inositol trisphosphate (IP_3) concentrations in vascular smooth muscle cells which, through a mobilization of IP_3 -sensitive intracellular calcium, results in contraction (Abdel-Latif, 1986). It is possible that an enhanced contractile response in vascular smooth muscle could be evoked by an enhanced accumulation of IP_3 resulting in an increased mobilization of intracellular calcium. The PPI transduction system is, there-

fore, an obvious target for study when mechanisms underlying postsynaptic changes responsible for the increased responsiveness of arteries from spontaneously hypertensive (SHR) rats as compared to arteries from normotensive (WKY) control rats are sought.

We have already reported an enhanced pressor response to nerve stimulation and exogenous noradrenaline in the tail artery from SHR rats compared to that from WKY rats (Muir & Wardle, 1989). Furthermore, we have shown that noradrenaline increased total IP accumulation, taken as a measure of PPI metabolism (Jenkinson *et al.*, 1990) in tail arteries from both WKY and SHR rats. However, no significant differences between results from SHR and WKY rats emerged. In a further attempt to explain the enhanced contractile responsiveness of tissues from hypertensive animals, we have measured the rates of formation, accumulation and metabolism of individual IPs, inositol monophosphate (IP_1), inositol biphosphate (IP_2) and IP_3 .

The study revealed no significant difference in the total accumulation of IPs or in the degree of breakdown of PPIs between SHR and WKY rats. There were significant differences, however, in the relative proportions of the individual IPs which constitute the total. Importantly, an enhanced accumulation of IP_3 in tissues from SHR rats was observed.

Methods

Preparation of arteries

Age-matched albino WKY and SHR rats (200–250 g) were killed by stunning followed by exsanguination. The tail was severed, the cornified epithelium removed and a length (7–8

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cm) of artery dissected out from the proximal end (Holman & Suprenant, 1980). Arteries were then cleaned of connective tissue and cut into segments (1 cm long, 5 mg wet wt). Changes in PPI metabolism in the segments were quantified by measuring IP accumulation (Akhtar & Abdel-Latif, 1984). To do this the segments were transferred to a glass vial containing a bicarbonate buffered salt solution (2 ml), of the following composition (mM): NaCl 118, NaHCO₃ 25, KCl 4, CaCl₂ 2.6, MgCl₂ 1.2, NaH₂PO₄ 1, glucose 11, pH 7.4, saturated with O₂/CO₂ (19:1), to which was added *myo*-[2-³H]-inositol (8 μ Ci ml⁻¹). This vial was incubated for 3 h at 37°C then the segments were transferred to another similar vial containing either an identical medium or one of the buffered salt solution supplemented with LiCl (10 mM) and *myo*-[2-³H]-inositol (8 μ Ci ml⁻¹). The time course of accumulation of IPs was measured both in the presence of LiCl, to inhibit the inositol monophosphatase and therefore the recycling of the IPs (Berridge, 1983), and in the absence of LiCl. These vials and contents were then incubated for a further 15 min at 37°C. After this preliminary incubation, segments were placed in other identical glass vials (3–4 segments per vial) at 37°C in Li-free or Li-containing medium and used in the following studies.

Effect and time course of noradrenaline on IP accumulation

The effects of noradrenaline (10⁻⁷–10⁻³ M) upon IP accumulation were examined 30 min after its addition to the Li-containing medium. The time course of accumulation of IPs was examined by incubating segments of artery for 1, 2, 5, 10, 20 or 30 min with or without 10⁻⁴ M noradrenaline in either Li-free or Li-containing bicarbonate buffer. The time course of the decay in the levels of IP accumulation, in response to 10⁻⁴ M noradrenaline, was investigated. The time course of metabolism of IPs was examined by the method of Batty & Nahorski (1989) which required that the incubations be carried out in the absence of LiCl (10 mM) to permit the recycling of the IPs unhindered by the inhibition of inositol monophosphatase by this salt. Segments were incubated in Li-free medium with 10⁻⁴ M noradrenaline for 30 min at which point the α_1 -adrenoceptor antagonist, prazosin (10⁻⁶ M; a concentration known to be effective in blocking both nerve- and exogenous noradrenaline-stimulated contraction of this tissue; Schwarze *et al.*, 1990), was added to terminate the stimulation of PPI metabolism by noradrenaline. Alternatively, in control vials, stimulation of PPI metabolism by noradrenaline was continued; controls received an equal volume of medium instead of prazosin. Segments were incubated for a further 1, 2, 5, 10, 15 or 20 min in this medium at 37°C. In all cases, incubations were terminated by transferring the segments to ice-cold trichloroacetic acid (TCA) (1 ml, 10% w/v).

Inositol phosphate extraction

TCA was extracted from the samples (1 ml) by repeated (3 times) addition and thorough mixing with water-saturated diethyl-ether. The pH of the samples was then adjusted to 7 with NaOH (0.2 ml, 0.1 M). The IPs were extracted and separated from the samples by anion exchange chromatography (Berridge, 1983) on Dowex 1-X8 anion exchange columns (100–200 mesh, formate form). Inositol trisphosphates (IP₃), bisphosphates (IP₂) and monophosphates (IP₁) were eluted separately. This was done by successive applications of (1) formic acid (0.1 M)/ammonium formate (0.2 M) solution to elute IP₁; (2) formic acid (0.1 M)/ammonium formate (0.4 M) solution to elute IP₂ and (3) formic acid (0.1 M)/ammonium formate (0.8 M) solution to elute IP₃. The amount of individual IPs and, when summated, total IP accumulation was thus measured. The eluted IPs were counted by liquid scintillation counting.

Measurement of systolic blood pressure

Systolic blood pressure was measured in conscious rats, acclimatized to 37°C for 15 min. Readings were made without anaesthesia by inflating a tail cuff; a piezo-electric crystal detector connected to a storage oscilloscope was used to determine when systolic blood pressure had been reached and a sphygmomanometer was used to measure blood pressure. The mean ($\pm$ s.e.mean) systolic blood pressure measured was: 125 $\pm$ 3.0 mmHg in WKY rats and 195 $\pm$ 1.9 mmHg in SHR rats (n = 10).

Analysis of results

Results are expressed as the mean $\pm$ standard error of mean (s.e.mean) of a number (n) of observations. Data were analysed by the Mann Whitney U-test. At t value of P < 0.05, or less, was taken as significant. * is used to denote P < 0.05; ** P < 0.01 and *** P < 0.001.

Drugs and chemicals

Myo-[2-³H]-inositol (18.3 Ci mmol⁻¹) in an aqueous ethanol solution (10% v/v) was purchased from Amersham International. ($\pm$)-Noradrenaline bitartrate was purchased from Sigma, ($\pm$)-prazosin hydrochloride was a gift from Pfizer Research, UK. Concentrations in text refer to the salts.

Results

Noradrenaline-stimulated inositol phosphate accumulation in arteries from WKY and SHR rats

Noradrenaline concentrations (10⁻⁶–10⁻³ M) which evoked contractions of this tissue (Schwarze *et al.*, 1990) evoked similar concentration-dependent, prazosin (10⁻⁶ M)-sensitive, increases in total IP accumulation in arteries from both SHR and WKY rats (Figure 1a). Analysis of the individual IPs, however, revealed differences between SHR and WKY rats. Noradrenaline (10⁻⁶–10⁻³ M) evoked a concentration-dependent increase in IP₁ (Figure 1b), IP₂ (Figure 1c) and IP₃ (Figure 1d) accumulation in both SHR and WKY rats with respect to controls. IP₁ accumulation was significantly greater in WKY rats at concentrations of noradrenaline of 10⁻⁶–10⁻⁴ M (Figure 1b). In the case of IP₂ (Figure 1c), no significant difference was observed between WKY and SHR populations except at the low concentrations of 10⁻⁷ M and 10⁻⁶ M at which IP₂ accumulation was significantly greater in WKY rats (Figure 1c). In contrast to the effects on IP₁ and IP₂, IP₃ accumulation was significantly greater in SHR than in WKY rats at all effective concentrations of noradrenaline (10⁻⁵–10⁻³ M) (Figure 1d).

Time course of noradrenaline (10⁻⁴ M)-stimulated inositol phosphate accumulation

The differences in the accumulation of individual IPs in response to noradrenaline (Figure 1) were investigated further by studying the time course of their accumulation. In the presence of LiCl, paralleling the conditions used in studies of the concentration-dependence of IP accumulation, 10⁻⁴ M noradrenaline stimulated a time-dependent accumulation of total IP in both SHR and WKY rats (Figure 2a). No significant difference emerged, at any time point measured, between tissues from SHR and WKY animals, confirming the results presented in Figure 1a. In contrast to measurements of total IP accumulation, 10⁻⁴ M noradrenaline-stimulated IP₁ accumulation in arteries from WKY rats was significantly greater after 20 min than in those from SHR rats (Figure 2b) consistent with the data in Figure 1b. Noradrenaline (10⁻⁴ M)-stimulated IP₂ accumulation was not different at any time point studied (Figure 2c) but accumulation of IP₃ at

every time point was significantly greater, including the zero time point (Figure 2d), in arteries from SHR than from WKY rats. These results are consistent with the data presented in Figure 1d.

Time course studies of the accumulation of the IPs were repeated in the absence of LiCl to confirm that (a) significant IP accumulation in response to noradrenaline could be

detected and that (b) differences in the accumulation of individual IPs persisted in the absence of the salt. Noradrenaline (10^{-4} M) stimulated a time-dependent, but significantly smaller, accumulation of total IP (Figure 3a), IP_1 (Figure 3b), IP_2 (Figure 3c) and IP_3 (Figure 3d) in the absence, compared with that in the presence, of LiCl. However, there were significant differences from the data presented in Figure 2.

Figure 1 The mean ($\pm$ s.e.mean, vertical bars, $n \geq 6$) increase in noradrenaline-evoked accumulation of total (a) and individual inositol phosphates (IPs), IP_1 (b), IP_2 (c) and IP_3 (d) expressed as disintegrations per minute (d.p.m.) mg^{-1} tissue wet weight (ordinate scale) in tail arteries from WKY (○) and SHR (Δ) rats compared with the respective controls (C). Noradrenaline evoked a concentration-dependent, prazosin (10^{-6} M)-sensitive (WKY, □; SHR, ■) increase in the accumulation of both total and individual IPs. This was significantly greater, for IP_3 (d), at concentrations of noradrenaline of 10^{-5} M and above in tissues from SHR compared to those from WKY rats. IP_1 levels (b) at 10^{-6} – 10^{-4} M noradrenaline were significantly greater in tissues from WKY than in those from SHR rats. There was no significant difference in total IP (a) or IP_2 (c) accumulation in response to noradrenaline between tissues from WKY and SHR rats.

Figure 2 The effect of time (abscissa scale, min) on the noradrenaline (10^{-4} M)-evoked accumulation (mean with s.e.mean, shown by vertical bars, $n \geq 6$) of total (a) and of individual inositol phosphates (IPs) (IP_1 (b), IP_2 (c), IP_3 (d)) expressed as disintegrations per minute (d.p.m.) mg^{-1} tissue wet wt (ordinate scale) in tail arteries from SHR (Δ) and WKY (○) rats compared with the respective controls (WKY, ●; SHR, ▲). LiCl was present throughout. The accumulation of IP_3 (d) in response to noradrenaline in arteries from SHR was greater than in those from WKY rats at each time point studied; for IP_2 (c), accumulation was not significantly different between the two populations and for IP_1 (b), accumulation was significantly greater in arteries from WKY rats after 20 min. Interestingly, control IP_3 (d) levels in SHR (▲) rats were significantly greater than in WKY (●) rats.

Noradrenaline (10^{-4} M) stimulated a significantly greater total IP accumulation, in incubations longer than 2 min, in SHR compared to WKY rats (Figure 3a); an effect not evident in the presence of LiCl. With the exception of the 30 min time point, no significant difference between IP_1 accumulation evoked by 10^{-4} M noradrenaline in SHR and WKY rats (Figure 3b) was observed. The difference in total

IP accumulation between SHR and WKY rats, observed in Figure 3a, is therefore due to differences in the accumulation of the other two IPs measured. IP_2 (Figure 3c) and IP_3 (Figure 3d)-accumulations in response to 10^{-4} M noradrenaline were greater at all time points studied (up to 30 min) in tissues from SHR than in those from WKY rats.

The decay of noradrenaline (10^{-4} M)-stimulated inositol phosphate accumulation

The declines in the accumulated total IP and individual constituent IPs were measured after addition of 10^{-6} M of the α_1 -adrenoceptor antagonist, prazosin, to terminate further PPI metabolism. In the absence of prazosin, noradrenaline (10^{-4} M)-stimulated IP accumulation remained constant throughout the 20 min period examined (Figure 4) confirming that metabolism of PPIs continued during this time.

Total inositol phosphate accumulation

In WKY rats, total IP accumulation in response to noradrenaline was greater than in non-stimulated controls (Figure 4a) at all time points studied. Within 5 min of the addition of 10^{-6} M prazosin, the levels of total IP were significantly lower than at time 0 but then no further decreases were observed. Similarly, noradrenaline-stimulated total IP accumulation in SHR rats declined with time from the addition of prazosin but remained significantly greater than in non-stimulated controls throughout the 20 min period studied. Total IP accumulation in SHR rats remained significantly greater than those values at equivalent time points in WKY rats until 15 min after addition of prazosin when total IP accumulation in tissues from both SHR and WKY rats were indistinguishable (Figure 4a). Prazosin alone had no effect upon basal total IP accumulation; basal levels were 611 ± 49 (SHR) and 580 ± 48 (WKY) d.p.m. mg^{-1} tissue (wet weight) in the absence and 812 ± 159 (SHR) and 622 ± 59 (WKY) d.p.m. mg^{-1} tissue (wet wt.) in the presence of prazosin.

Inositol monophosphate accumulation

Noradrenaline-induced increases in IP_1 levels were similar in both WKY and SHR rats and declined with time, but remained significantly above those in non-stimulated controls, throughout the 20 min period following the addition of prazosin (Figure 4b) to arrest stimulation. However, the rate at which IP_1 levels declined differed between the two groups, being greater in WKY rats. This resulted in IP_1 levels in SHR rats remaining significantly greater than those at equivalent time points in WKY rats for up to 10 min; after this time values in the two groups were indistinguishable (Figure 4b). Prazosin alone had no effect upon basal IP_1 accumulation; basal levels were: 184 ± 21 (SHR) and 181 ± 11 (WKY) d.p.m. mg^{-1} tissue (wet wt.) in the absence and 189 ± 24 (SHR) and 204 ± 21 (WKY) d.p.m. mg^{-1} tissue (wet wt.) in the presence of prazosin.

Inositol bisphosphate accumulation

Although IP_2 levels decayed with time in the presence of prazosin, in both WKY and SHR rats (Figure 4c), they remained significantly greater than the respective non-stimulated controls at all time points examined. In SHR rats, noradrenaline-stimulated IP_2 accumulation initially increased within 1 min of the addition of prazosin and remained significantly elevated compared to values in WKY tissues for 10 min after the addition of the antagonist (Figure 4c). Prazosin alone had no effect upon basal IP_2 accumulation; basal levels were: 129 ± 6 (SHR) and 114 ± 6 (WKY) d.p.m. mg^{-1} tissue (wet wt.) in the absence and 147 ± 33 (SHR) and 129 ± 15 (WKY) d.p.m. mg^{-1} tissue (wet wt.) in the presence of prazosin.

Figure 3 The effect of time on the noradrenaline (10^{-4} M)-evoked accumulation (mean with s.e.mean, shown by vertical bars, $n \geq 6$) of total inositol phosphates (IP) (a) and individual IPs (IP_1 (b), IP_2 (c), IP_3 (d)) expressed as disintegrations per minute (d.p.m.) mg^{-1} tissue wet wt (ordinate scale) in tail arteries from SHR (Δ) and WKY ($\circ$) rats compared with the respective controls (SHR, Δ ; WKY, $\bullet$). LiCl was absent. The accumulation of IPs was significantly smaller than in the presence of LiCl (c.f. Figure 2). Accumulation of total IP (a), IP_2 (c), and IP_3 (d) was significantly greater in tissues from SHR than in those from WKY rats where indicated. Noradrenaline-evoked accumulation of IP_1 (b) in tissues from SHR and WKY rats did not differ significantly.

Figure 4 The decay of noradrenaline (10^{-4} M)-stimulated inositol phosphate (IP) accumulation (mean with s.e.mean, shown by vertical bars, $n \geq 6$) of total (a) and individual IPs (IP₁ (b), IP₂ (c) and IP₃ (d)) with time (abscissa scale, min) in tail arteries from SHR (Δ) and WKY (○) rats in the presence of prazosin (10^{-6} M, time 0) to arrest the effects of noradrenaline. IP accumulations in the absence of prazosin are represented thus (SHR, ▲; WKY, ●). In all cases IP levels remained above basal (----) levels.

Inositol trisphosphate accumulation

IP₃ accumulation in response to noradrenaline was higher in WKY rats than in non-stimulated controls (Figure 4d) but, in contrast to that of the other IPs, did not decline significantly with time after the addition of 10^{-6} M prazosin. In contrast, noradrenaline-stimulated IP₃ levels in SHR rats declined from the time of addition of the antagonist, but remained significantly greater than in the non-stimulated controls throughout the 20 min period investigated. This decline took place from a significantly greater level in SHR rats, compared to that at the equivalent time point in tissues from

WKY rats. IP₃ levels remained significantly greater than those values at equivalent time points in WKY rats until 10 min after addition of prazosin at which point IP₃ levels in tissues from both SHR and WKY rats were indistinguishable. Prazosin alone had no effect upon basal IP₃ accumulation; basal levels were 298 ± 22 (SHR) and 285 ± 30 (WKY) d.p.m. mg⁻¹ tissue (wet wt.) in the absence and 476 ± 101 (SHR) and 290 ± 24 (WKY) d.p.m. mg⁻¹ tissue (wet wt.) in the presence of prazosin.

Discussion

Measurement of total IP accumulation, as an indication of alterations in the PPI signal-transduction pathway, can be misleading. The lack of difference in total IP accumulation measured in the presence of LiCl contrasted with the significant differences in the accumulation of individual IPs between SHR and WKY rats. Significantly greater levels of IP₃ accumulated, at each effective concentration of noradrenaline, in tissues from SHR compared to those from WKY rats. This was paralleled by a reduction in IP₁ accumulation and no significant differences in IP₂ accumulation, consistent with the lack of a significant difference in noradrenaline-stimulated total IP accumulation between the two populations. Since IP₃ is the PPI product responsible for calcium mobilization (Berridge & Irvine, 1989), its enhanced accumulation could account for the increased contractility of tissues from SHR rats. Interestingly, basal IP₃ level were found to be significantly greater in arteries from SHR than in WKY rats and may be a contribution to the increased contractility of such tissues. The measured accumulation of IP₃ reflects the liberation of IP₃ from PIP₂, the net metabolism of IP₃ to IP₂ and the formation of IP₃ from higher phosphorylated IPs such as inositol tetrakisphosphate (IP₄), inositol pentakisphosphate (IP₅) and inositol hexakisphosphate (IP₆). (1,4,5)-IP₃ can be further phosphorylated to (1,3,4,5)IP₄ which is then dephosphorylated to (1,3,4)IP₃ and further rephosphorylated to (1,3,4,5)IP₄ (for review see Berridge & Irvine, 1989). Of the two major IP₃ isomers (1,4,5)IP₃ and (1,3,4)IP₃, only the former is responsible for the liberation of calcium from internal stores (Berridge & Irvine, 1989). (1,3,4)IP₃ is formed by the dephosphorylation of (1,3,4,5)IP₄ and is an intermediary in the recycling of inositol back into the PPIs. Since anion exchange chromatography does not permit the relative proportions of the two isomers in the IP₃ fraction to be resolved, it cannot be determined whether or not the increased accumulation of IP₃ in arteries from SHR rats is of the important calcium mobilizing (1,4,5)IP₃ isomer.

The lack of difference in total IP accumulation between tissues from SHR and WKY rats indicates that the extent of noradrenaline-induced stimulation of phospholipase C (PLC) in tissues from SHR and WKY rats may have been similar. This implies that the enhanced accumulation of IP₃ in tissues from SHR rats is due to differences in the subsequent metabolism of IPs following their liberation from PPIs. From time course studies, the reason for the enhanced noradrenaline-induced accumulation of IP₃ in arteries from SHR rats may be a reduced rate of dephosphorylation of IP₃.

In the presence of LiCl, the rate of noradrenaline-stimulated IP₃ accumulation was greater in SHR rats from the moment of addition of the catecholamine. This was matched by a corresponding decrease in the rate of IP₁ accumulation resulting in no net difference in total IP accumulation between SHR and WKY rats over the time period studied. Studies on the metabolism of IPs are complicated by the presence of LiCl. This salt is used to block recycling of inositol back to PPIs and so facilitates measurement of IPs (Berridge, 1983). However, this results in the successive accumulation of IP₁ and IP₂ which will produce an inhibition of the IP₂ and IP₃ phosphatases as accumulation of these products occurs (Shears *et al.*, 1987). It is therefore difficult to separate changes in the rate of metabolism of IPs by

phosphatases from those which arise from the altered sensitivity of these enzymes to inhibition by accumulation of reaction products (IP₁, IP₂ and IP₃). These problems are absent when LiCl is removed. Interestingly, in the absence of LiCl, significant differences were observed in the time course of noradrenaline-stimulated total IP accumulation, an observation masked in studies performed in the presence of the salt. In the absence of LiCl, the accumulation of IP₁ was reduced to about 5% of that seen in the presence of LiCl and no differences were evident between the levels of this IP in either SHR or WKY rats. However, a substantial accumulation of IP₃ in both SHR and WKY rats remained and this was enhanced by noradrenaline to a greater extent in tissues from SHR rats than in those from WKY rats. An enhanced accumulation of IP₂ in response to noradrenaline in SHR rats was also revealed. The lack of difference in net PPI metabolism (and therefore IP liberation) between SHR and WKY rats, compatible with an enhanced accumulation of IP₂ and IP₃ but not IP₁ in arteries from SHR rats, may arise from a reduced metabolism of IP₃ to IP₂ to IP₁ in these arteries.

We attempted to investigate differences in the rates of metabolism of IPs between arteries from SHR and WKY rats by measuring the rates of decay of the individual IPs after the termination of the hydrolysis of PPIs. This was achieved by the addition of the α_1 -adrenoceptor antagonist, prazosin, which itself had no effect upon basal levels of any of the IPs measured. The rates of decay in the levels of the IPs, in particular the rates of decay of IP₃, may reveal differences in the metabolism of IPs between the two groups but unfortunately, measuring the time course of the decays in the levels of IPs did not reveal any differences. IP₃ levels declined after the addition of prazosin in SHR rats from an initially greater value and remained significantly greater than in WKY rats. However, any comparison with the rate of decay of IP₃ levels in WKY rats was impossible as IP₃ levels in

tissues from WKY rats did not decline significantly after addition of prazosin. This could have been due to insufficient prazosin being added to terminate noradrenaline-stimulation of PPI hydrolysis, but is unlikely as 10^{-6} M prazosin completely blocked the contraction and IP accumulation stimulated by this concentration of noradrenaline in these arteries (Schwarze *et al.*, 1990). One possible explanation is that changes in the specific activity of the ³H-labelled-product may be responsible for the lack of decay of IP₃ after the addition of prazosin in WKY rats, as changes in specific radioactivity are revealed in that fraction of the response which is not reversible by antagonist (Batty & Nahorski, 1989). However, the apparent steady state of IP₃ levels is most likely to be a reflection of the complicated, and as yet ill-defined, metabolism of IPs which may result in a constant metabolism and formation of IP₃s via various routes. It may require the resolution of the isomers of the IPs and the additional measurement of more highly phosphorylated IPs (e.g. IP₄, IP₅ and IP₆) before more meaningful results are obtained from the measurement of the metabolism of IPs in these tissues.

In conclusion, this study suggests that, in tail arteries of SHR rats, there is an altered signal-transduction mechanism which mediates noradrenaline-evoked contractions and which results in an enhanced accumulation of IP₃. This change may be an altered metabolism of IPs resulting in accumulation of more of the calcium-releasing IP₃. Although the particular isomer of IP₃ was not identified, it is tempting to speculate that the enhanced accumulation of IP₃ may result in an increased calcium mobilization and so account for the increasing contractility to noradrenaline.

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