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Existence and α_1-Adrenergic Stimulation of Inositol Polyphosphates in Mammalian Heart

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SUMMARY

The concentration-response curves and the time course of the effects of phenylephrine (0.01–100 μM) on force of contraction and on inositol polyphosphates in isolated electrically stimulated perfused rat hearts (Langendorff technique) were studied. A nonradiometric high performance liquid chromatography metal dye detection technique was used to determine absolute concentration masses/changes of inositol polyphosphates in heart. Products measured after separation with high performance liquid chromatography were inositol 1,4,5-trisphosphate (1,4,5-IP3), inositol 1,3,4,5-tetrakisphosphate (1,3,4,5-IP4) and its isomer 1,3,4,6-IP4, inositol 1,3,4,5,6-pentakisphosphate (1,3,4,5,6-IP5), and inositol hexakisphosphate (IP6). 1,4,5-IP3 (significant at 10 μM) and both IP4 isomers (significant at 1 μM) increased after α-adrenoceptor stimulation, whereas 1,3,4,5,6-IP5 and IP6 remained unaffected. Phenylephrine had a concentration-dependent positive inotropic effect (significant at 1 μM). All effects were antagonized by the α_1-adrenoceptor antagonist prazosin (0.1 μM), indicating receptor-mediated effects. In a time course study 1,4,5-IP3 was the first compound to increase significantly, within 1 min after stimulation; this rise was followed by an increase in 1,3,4,5-IP4 beginning within 2 min. The increase in all other inositol polyphosphates was slower (5–10 min). The increase in the force of contraction started at 2 min. For comparison, the effects of the β-adrenoceptor agonist isoprenaline were studied. Isoprenaline produced a positive inotropic effect similar to that of phenylephrine, but all inositol polyphosphates remained unaffected. In conclusion, for the first time the existence of 1,3,4,5,6-IP5 and IP6 was observed in the heart. However, the physiological role of these inositol polyphosphate isomers in the heart remains to be elucidated, because, from the time course, they appear to have no acute intracellular second messenger function. Increased inositol polyphosphate turnover may be involved in the mechanism(s) whereby α_1-adrenoceptor stimulation produces an increase in myocardial force of contraction. Because the increase in 1,4,5-IP3 precedes and that in 1,3,4,5-IP4 coincides with the increase in the force of contraction, 1,4,5-IP3 may initiate and 1,3,4,5-IP4 may maintain the positive inotropic effect of α_1-adrenoceptor agonists.

Although the catecholamine-induced increase in force of contraction in the heart is mainly due to stimulation of myocardial β-adrenoceptors, the existence of α_1-adrenoceptors mediating positive inotropic effects, but not chronotropic effects, in the mammalian heart is well established. More recently, evidence has been accumulating that the second messenger 1,4,5-IP3 is involved in α_1-adrenoceptor-mediated positive inotropic effects in the heart of rats and humans (1–4). Stimulation of α_1-adrenoceptors activates phospholipase C, via a guanine nucleotide-binding protein; phospholipase C then hydrolyzes phosphatidylinositol 4,5-bisphosphate and generates the second messengers diacylglycerol and 1,4,5-IP3 (5, 6). The guanine nucleotide-binding protein involved in phospholipase C activation in the heart is of as yet unknown nature (7, 8). Diacylglycerol activates protein kinase C. The functional role of protein kinase C in heart muscle contraction is not as yet well understood (9). However, protein kinase C activators have been shown to activate voltage-dependent cardiac calcium channels (10). 1,4,5-IP3 releases calcium from intracellular stores in a variety of cell systems, including smooth muscle (5). In cardiac muscle, some authors failed to observe an 1,4,5-IP3-induced calcium release from sarcoplasmic reticulum (11), although there is evidence that 1,4,5-IP3 is, indeed, an intracellular calcium-mobilizing agent in cardiac muscle and that 1,4,5-IP3 can thereby increase the force of contraction (12–15).

In addition to 1,4,5-IP3, its phosphorylation product 1,3,4,5-IP4 has also been claimed to possess second messenger function (16). 1,3,4,5-IP4 can be dephosphorylated to 1,3,4-IP3 (17, 18). The existence of both isomers in the heart has been shown recently (19, 20). Moreover, 1,3,4-IP3 can be phosphorylated to another IP4 isomer, 1,3,4,6-IP4 (21–23). This IP4 isomer has been shown to be a precursor of 1,3,4,5,6-IP5 (24). Whether 1,3,4,5,6-IP5 is likely to be the precursor of IP6 is still a matter of debate (5, 25).

In the heart, the existence and the *in vivo* content of these inositol polyphosphates are as yet unknown, and their identification requires a highly resolving chromatographic system. Therefore, a recently developed HPLC technique using metal

ABBREVIATIONS: IP3, inositol trisphosphate; IP4, inositol tetrakisphosphate; IP5, inositol pentakisphosphate; IP6, inositol hexakisphosphate; HPLC, high performance liquid chromatography; PAR, 4-(2-pyridylazo)resorcinol.

dye detection, which permits picomolar range analysis of inositol polyphosphates from tissues that are not radioactively labeled (26), was used. The purpose of this study was to evaluate the concentration- and time-dependent effects of phenylephrine on different products of inositol lipid metabolism and on the force of contraction in the heart.

Materials and Methods

Isolated perfused hearts. The experiments were performed on isolated electrically driven (frequency, 5 Hz; duration, 5 msec; intensity, 20% greater than threshold) hearts of male Wiga rats (body weight, 250–320 g). The animals were pretreated with reserpine (5 mg/kg intraperitoneally, 18 hr before death), to exclude the influence of endogenous catecholamines. To prevent the formation of thrombi in the coronary vessels, all animals received heparin (20 mg/kg intraperitoneally) 30 min before the experiments were started. The animals were killed by a blow to the head and were bled from the carotid arteries. The hearts (wet weight, 718–1013 mg) were quickly excised, attached to a glass canula in a aerated (95% O_2/5% CO_2) perfusion solution (see below) at room temperature, mounted on a double-barrelled Langendorff perfusion apparatus, and immediately perfused through the aorta at a constant pressure of 60 cm of H_2O, as described previously (27). The perfusion solution, which was a modified Tyrode solution containing (in mM) 119.8 NaCl, 1.8 $CaCl_2$, 5.4 KCl, 1.05 $MgCl_2$, 0.42 NaH_2PO_4, 22.6 $NaHCO_3$, 0.05 Na_2EDTA, 0.28 ascorbic acid, and 5.0 glucose, was continuously gassed with 95% O_2/5% CO_2 and maintained at 35°, with a pH of 7.4. The force of contraction was measured according to the method of Beckett (28), with an inductive force transducer (W. Fleck, Mainz, FRG), and was recorded on a Hellige Helco Scriptor recorder. The diastolic tension was kept constant throughout the experiments. The hearts were allowed to beat during drug-free perfusion until the force of contraction had reached equilibrium (at least 30 min).

The force of contraction was measured in the following manner. After mechanical stabilization, the hearts were perfused for 30 min with 1 μM propranolol, to avoid any interference from β-adrenergic stimulation, and, where used, the α-adrenoceptor antagonist prazosin (0.1 μM) was also applied. Thereafter, phenylephrine (0.01–100 μM) or, for comparison, isoprenaline (10 μM) was perfused as indicated and was present for 15 min, except for the experiments to evaluate the time course (0–15 min). Each heart was exposed to one drug concentration only. At the end of each experiment, the right and left ventricles were frozen in liquid nitrogen for determination of inositol phosphates.

Inositol phosphates. The preparations were prepared for HPLC metal dye detection analysis in three steps, by a $HClO_4$ extraction, a charcoal treatment, and a solid-phase extraction, as described previously (26). In brief, the frozen ventricles were powdered in a liquid nitrogen-cooled microdismembrator (Braun, Melsungen, FRG) and extracted by addition of $HClO_4$. After centrifugation, the supernatant was adjusted to a pH of 5 by addition of KOH, and the $KClO_4$ precipitate was removed by centrifugation. Thereafter, the samples were frozen, freeze-dried, and redissolved for a charcoal treatment. A suspension of acid-treated Norit A was added to the samples, samples were vortex-mixed and centrifuged, and the supernatants were treated with charcoal once more. The two charcoal pellets were consecutively reextracted with 0.1 M NaCl, and the supernatant was combined with the sample. For solid-phase extraction, the samples were diluted with water and applied to a Q-Sepharose column (chloride form). After washing with 2 mM HCl, the inositol polyphosphates were eluted with 0.54 M HCl. This eluate was frozen, freeze-dried, and redissolved for HPLC analysis.

An inert HPLC system from Pharmacia, with two 2248 HPLC pumps, a 2252 LC controller, a 2151 UV detector (254 nm), a UV-M monitor (546 nm), a 2157 autosampler, and two Mono Q columns (0.5 × 5 cm and 0.5 × 20 cm), was used. The absorbance signals from both detectors were integrated by a two-channel integrator (Nelson chromatography system). The gradient was mixed at a flow rate of 1 ml/min, using eluents A (0.2 mM HCl plus 14 μM YCl_3) and B (0.5 M HCl plus 14 μM YCl_3). The post-column-added dye solution had a flow rate of 0.5 ml/min and contained 300 μM PAR plus 1.6 M triethanolamine, adjusted to pH 9.0 with HCl. The gradient was run from 4% eluent B to 100% eluent B in 80 min.

A typical HPLC metal dye detection analysis is shown in Fig. 1. Inositol phosphates measured were 1,4,5-IP3, 1,5,6-IP3, 4,5,6-IP3, 1,3,4,6-IP4, 1,3,4,5-IP4, 2,4,5,6-IP4, 1,2,4,5,6-IP5, 1,3,4,5,6-IP5, and IP6. The identities of inositol polyphosphates were determined by coelution with D-*myo*-inositol standards (NMR analyzed). Additional structural characterization of the peaks has not been performed, and the peaks could contain more than one isomer (see Discussion). Because, at present, the number of inositol phosphate standards available is still much smaller than the number of isomers known or theoretically possible, there were more positive signals detected than assigned. However, some peaks in Fig. 1 were assigned from partly hydrolyzed phytic acid, according to the method of Mayr (26). Recovery of 1,4,5-IP3 was 90.5 ± 7.5%, of 1,3,4,6-IP4 was 91.0 ± 8.2%, of 1,3,4,5-IP4 was 89.1 ± 8.2%, of 1,3,4,5,6-IP5 was 88.8 ± 7.6%, and of IP6 was 89.3 ± 8.4% (eight experiments each).

Drugs. Substances used were (−)-phenylephrine-HCl, (±)-isoprenaline-HCl (both from Boehringer, Ingelheim, FRG), prazosin-HCl (Pfizer, Karlsruhe, FRG), (±)-propranolol-HCl (Rheinpharma, Heidelberg, FRG), D-*myo*-1,4,5-IP3, D-*myo*-1,3,4,6-IP4, D-*myo*-1,3,4,5-IP4, D-*myo*-1,3,4,5,6-IP5 (all from Boehringer, Mannheim, FRG), IP6 (Sigma, St. Louis, MO), Titrisol-HCl, perchloric acid (both from Merck, Darmstadt, FRG), Norit A (Serva, Heidelberg, FRG) (further treated by boiling for 2 hr in 3 M HCl, washing with water to neutrality, and drying at 120°), Q-Sepharose fast flow (Pharmacia, Uppsala, Sweden), yttrium trichloride hexahydrate (Janssen, Beerse, Belgium), PAR (Serva, Heidelberg, FRG), and triethanolamine (Fluka, Buchs, Switzerland). All other chemicals were of analytical grade or the best grade commercially available. All substances were freshly dissolved in prewarmed and pregassed bathing solution. Deionized and twice-distilled water was used throughout.

Statistics. The values presented are means ± standard errors. Statistical significance was estimated with Student's *t* test for unpaired observations. A *p* value of <0.05 was considered significant.

Results

Concentration-dependent effects. The effects of the α_1-adrenoceptor agonist phenylephrine (10 μM) are summarized in Table 1. Phenylephrine increased the force of contraction to 192% of the predrug value and increased several but not all inositol polyphosphates. Inositol phosphate products measured after separation by HPLC were 1,4,5-IP3, 1,3,4,6-IP4, 1,3,4,5-IP4, 1,3,4,5,6-IP5, and IP6. 1,4,5-IP3 and both IP4 isomers increased after α_1-adrenoceptor stimulation, whereas 1,3,4,5,6-IP5 and IP6 remained unaffected. Of all inositol polyphosphates, 1,4,5-IP3 had the highest concentration. The effects of phenylephrine on inositol phosphates and the force of contraction were blocked by the α_1-adrenoceptor antagonist prazosin (0.1 μM) (Table 1). The β-adrenoceptor agonist isoprenaline (10 μM), which also was investigated in the presence of propranolol (1 μM) in order to establish identical conditions, produced a similar increase in the force of contraction, to 200% of the predrug value, but all inositol polyphosphates measured remained unchanged (Table 1).

Fig. 2, A to E, shows the concentration-response curves for the phenylephrine-induced effects on inositol polyphosphates. Accumulation of 1,4,5-IP3 (Fig. 2A) began at 10 μM phenylephrine and for both IP4 isomers (Fig. 2, B and C) at 1 μM

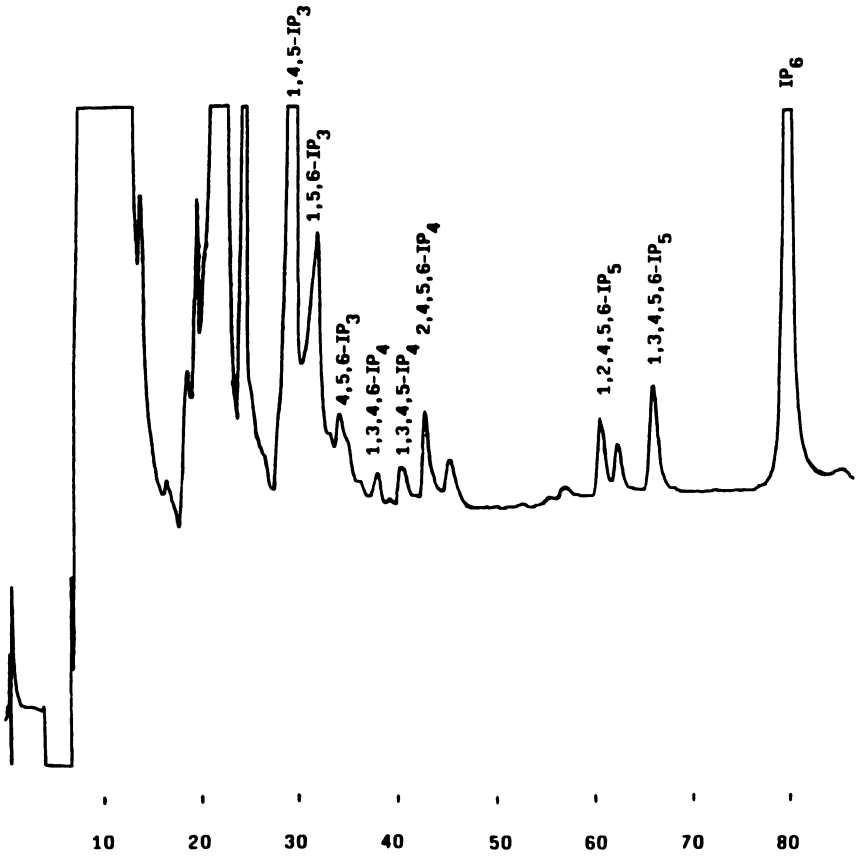

Fig. 1. Typical HPLC metal dye detection analysis of the inositol polyphosphate content from rat hearts in the presence of phenylephrine (10 μM; 5 min).

TABLE 1

Effects of α_1- or β-adrenoceptor stimulation on inositol phosphate content and on force of contraction

The isolated perfused rat hearts were stimulated electrically and analyzed as described in Materials and Methods. Values are means ± standard errors, in pmol/mg of wet weight (inositol phosphates) or percentage of predrug value (force of contraction). All experiments were performed in the presence of propranolol (1 μM) and, where used, prazosin (0.1 μM) was also applied. The incubation time for phenylephrine (10 μM) and isoprenaline (10 μM) was 15 min. All five inositol phosphates were measured in each heart. The predrug value of force of contraction was 99.5 ± 4.5 mN (28 experiments).

	Control ($n = 7$)[a]	Phenylephrine ($n = 7$)	Phenylephrine + prazosin ($n = 7$)	Isoprenaline ($n = 7$)
Inositol phosphates (pmol/mg)				
1,4,5-IP3	7.40 ± 0.5	13.2 ± 0.60[b]	6.90 ± 0.5	7.20 ± 0.7
1,3,4,6-IP4	0.34 ± 0.05	0.72 ± 0.12[b]	0.31 ± 0.07	0.35 ± 0.08
1,3,4,5-IP4	0.10 ± 0.01	0.21 ± 0.02[b]	0.11 ± 0.01	0.09 ± 0.01
1,3,4,5,6-IP5	0.20 ± 0.05	0.25 ± 0.05	0.23 ± 0.06	0.21 ± 0.04
IP6	1.00 ± 0.5	1.50 ± 0.8	1.20 ± 0.6	0.90 ± 0.7
Force (%)	99.0 ± 1.0	192.0 ± 10.0[b]	106.0 ± 6.0	200.0 ± 11.0[b]

[a] n = number of experiments.
[b] $p < 0.05$, versus control.

phenylephrine. At 100 μM phenylephrine, the highest concentration investigated, the effects on 1,3,4,5-IP4 and 1,3,4,6-IP4 content apparently did not reach a maximum. 1,3,4,5,6-IP5 and IP6 (Fig. 2, D and E) remained unchanged under all conditions. The concentration dependence of the effects of phenylephrine on the force of contraction is shown in Fig. 2F. The positive inotropic effect began at 1 μM phenylephrine, and at 100 μM phenylephrine the effect did not reach a clear maximum.

Time-dependent effects. The time course of inositol polyphosphate content in the absence and presence of phenylephrine (10 μM) is shown in Fig. 3, A to E. A significant increase in 1,4,5-IP3 could already be detected at 1 min, reached maximum at 10 min, and remained constant thereafter (Fig. 3A). 1,3,4,6-IP4 became significant at 5 min and remained constant thereafter (Fig. 3B). 1,3,4,5-IP4 could be detected at 2 min, was maximal at 5 min, and remained nearly constant thereafter (Fig. 3C). 1,3,4,5,6-IP5 became significant at 10 min and IP6 at 5 and 10 min (Fig. 3, D and E), followed by a decrease to control levels within 15 min. Fig. 3F shows the time course of the force of contraction in the absence and presence of phenylephrine. An increase in the force of contraction was not seen before 2 min and reached maximum after 5 min.

Discussion

The present study shows a previously unknown content of inositol polyphosphates in the heart, i.e., 1,4,5-IP3, 1,3,4,6-IP4, 1,3,4,5-IP4, 1,3,4,5,6-IP5, and IP6. The increase in 1,4,5-IP3 precedes and that in 1,3,4,5-IP4 coincides with the positive inotropic effect after α_1-adrenoceptor stimulation. This provides evidence that 1,4,5-IP3 may initiate and 1,3,4,5-IP4 maintains the positive inotropic effect.

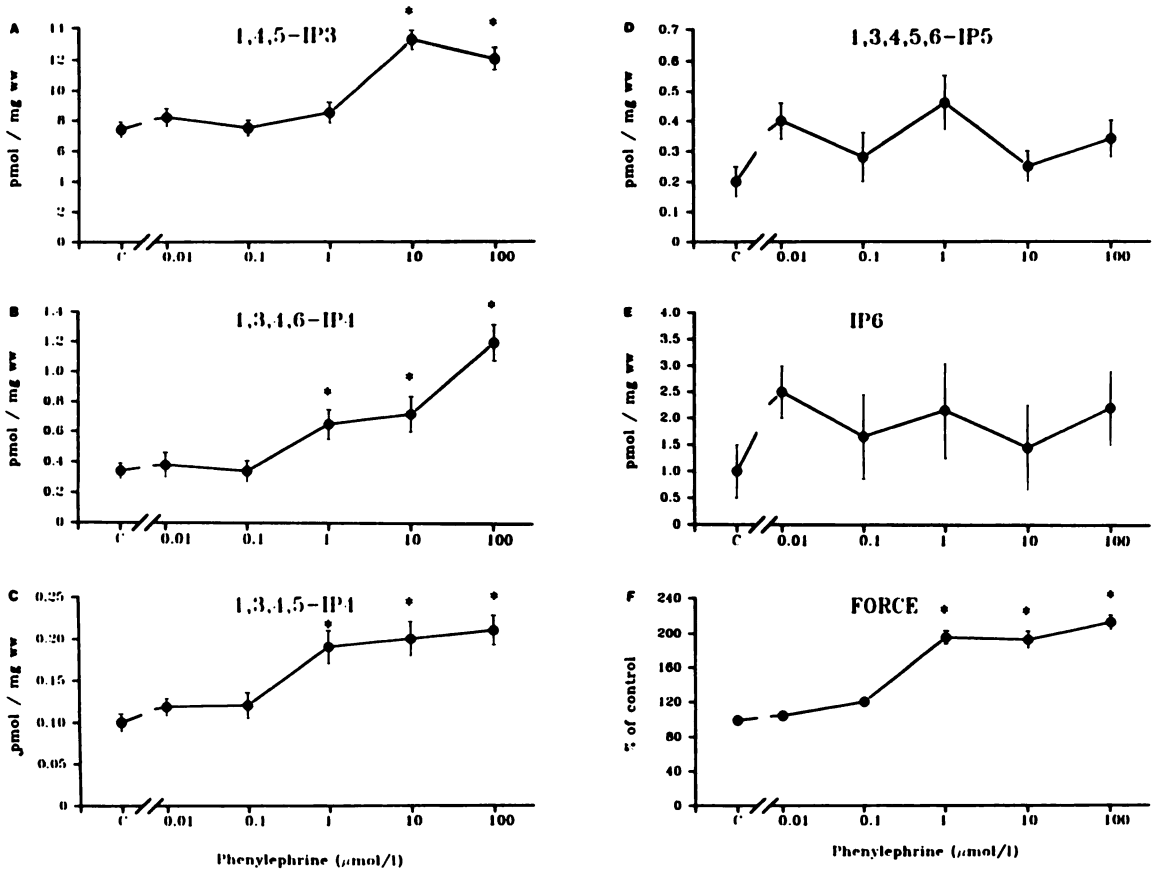

Fig. 2. Concentration-response curves for the effects of phenylephrine on inositol polyphosphates (A–E) or force of contraction (F) of isolated, electrically driven, perfused rat hearts in the presence of propranolol (1 μM). Phenylephrine was applied 30 min after addition of propranolol and was present for 15 min. *Ordinates*, inositol phosphate content, in pmol/mg of wet weight, and force of contraction, as percentage of predrug value. *Abscissae*, concentration of phenylephrine, in μM. The predrug value for the force of contraction was 107.1 ± 2.65 mN (42 experiments). The number of experiments was seven for each point. *, $p < 0.05$, versus control (*C*).

Stimulation of α_1-adrenoceptors in the heart leads to a positive inotropic effect, which has been explained by an increase in slow calcium inward current (29) and an increase in calcium sensitivity of contractile proteins (30). Moreover, the positive inotropic effect has been attributed to an increased inositol phosphate turnover (1–3). However, Woodcock *et al.* (31), using HPLC, detected 1,4,5-IP3 but not 1,3,4,5-IP4 and concluded that the IP3/IP4 pathway does not exist in the heart. We and others have recently determined 1,3,4,5-IP4 in the heart (19, 20). Thus, the present study supports the existence of the IP3/IP4 pathway in the heart.

In other tissues, inositol polyphosphates are associated with two functional groups. One is the agonist-sensitive group, whose levels change in response to agonist stimulation and which may have functions related to intracellular signaling. The second is the agonist-insensitive group, whose levels, if they change, do so comparatively slowly during stimulation (5). This view is supported in the present study. 1,4,5-IP3 and both IP4 isomers show concentration-dependent effects, whereas 1,3,4,5,6-IP5 and IP6 remained unchanged, regarding the concentration-dependent effect. In the time course study, 1,3,4,5,6-IP5 and IP6 revealed a slow and transitory increase, which decreased to control levels within 15 min. Thus, these compounds fit into the agonist-insensitive group, which has no acute intracellular second messenger function, although there seems to be a pathway from the agonist-sensitive to the agonist-insensitive group (see below), which could explain the transient increase. However, the increase in 1,4,5-IP3 preceded and that in 1,3,4,5-IP4 coincided with the α_1-adrenoceptor-mediated positive inotropic effect. All other inositol polyphosphates increased after the increase in the force of contraction. Thus, an important prerequisite for a causal relation between positive inotropic effect and 1,4,5-IP3 increase is fulfilled; the former preceded the latter. The increase in 1,3,4,5-IP4 was slower, which may be explained by the phosphorylation of 1,4,5-IP3 to form 1,3,4,5-IP4. 1,3,4,5-IP4 has been suggested to have second messenger function too, i.e., to initiate calcium entry from the exterior (16) and/or to control the transfer of calcium between intracellular pools (5). The present time courses are consistent with previous data (4) and support the theory that the initial phase might be due to formation of 1,4,5-IP3 and that 1,3,4,5-IP4 might be responsible for the maintenance of the positive inotropic effect. Moreover, stimulation of α_1-adrenoceptors increased the force of contraction and 1,4,5-IP3, as well as 1,3,4,5-IP4, to approximately the same extent, and all effects were blocked by the α_1-adrenoceptor antagonist prazosin, indicating receptor-mediated effects.

Although IP5 and IP6 have been suggested to have extracellular second messenger function (32), they are generally assumed to have no acute intracellular second messenger function (5). IP5 and IP6 have been shown to exist in amoebae, plants, fish, amphibians, and birds, and they are also present in mam-

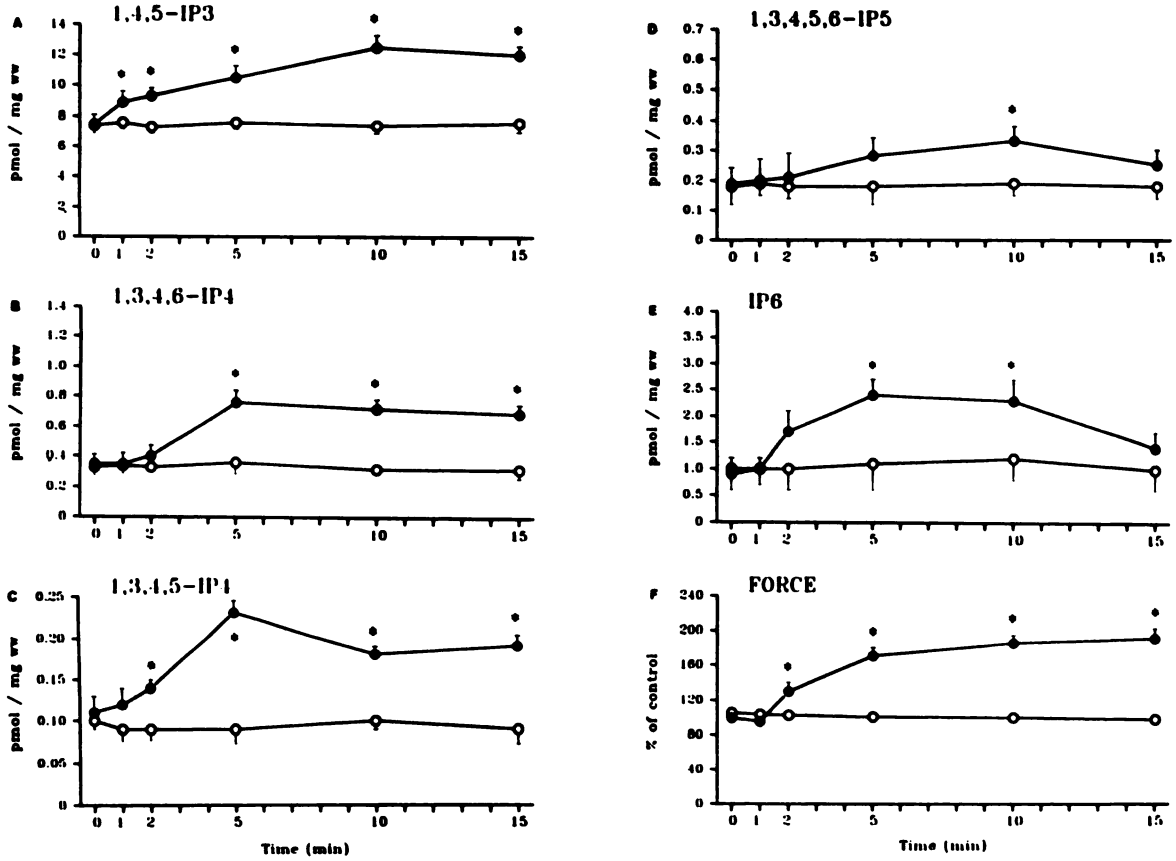

Fig. 3. Time course of inositol polyphosphates (A-E) or force of contraction (F) of isolated, electrically driven, perfused rat hearts, in the absence (○) and presence (●) of phenylephrine (10 μM). All experiments were performed in the presence of propranolol (1 μM). Phenylephrine was applied at zero time, 30 min after addition of propranolol. *Ordinates*, inositol phosphate content, in pmol/mg of wet weight, and force of contraction, as percentage of predrug value. *Abscissae*, time of incubation with phenylephrine, in min. The values for the force of contraction at zero time were 109.1 ± 5.8 mN (○) (42 experiments) and 98.8 ± 5.0 mN (●) (42 experiments). The number of experiments was seven for each point. *, $p < 0.05$, versus predrug value.

malian tissues such as cerebral cells, salivary glands, and oocytes (25, 33), yet little is known about their cellular functions or about their intracellular pathways. However, IP5 and IP6 appear to have specific physiological functions, e.g., in birds, they modulate the oxygen affinity of hemoglobin in a manner similar to that of 2,3-diphosphoglycerate in mammalian red blood cells (34). In the case of 1,3,4,5,6-IP5, a pathway exists from the agonist-sensitive inositol phosphates, from 1,4,5-IP3 to 1,3,4,5-IP4 to 1,3,4-IP3 to 1,3,4,6-IP4, and, hence, to the agonist-insensitive inositol polyphosphate 1,3,4,5,6-IP5 (24, 35). Recently, it has been demonstrated in oocytes that IP5 is a precursor of IP6 (25). However, IP5 is slowly converted to IP6. Only 30% of the IP5 was phosphorylated to IP6 within 72 hr, indicating that these inositol phosphates do not function as acute intracellular messengers. One potential physiological role for IP5 and IP6 may involve long term modulatory processes, such as trophic regulation or cell adaption to sustained agonist activation (25). The present study shows that, except for 1,4,5-IP3 and 1,3,4,5-IP4, there is no temporal correlation between increase in force of contraction and accumulation of all other inositol polyphosphates measured. Thus, in the heart these compounds are probably also excluded as acute intracellular messengers in receptor-mediated increases in the force of contraction.

There is difficulty in interpreting data from isotope studies, because of the inadequate labeling of some of the metabolic pools. This has focused interest on the measurements of unlabeled inositol phosphates. In this study a novel HPLC metal dye detection method was used for mass measurement of unlabeled inositol phosphates (26). Metal dye detection involves the competition of inositol phosphates with a cation-specific dye (PAR) for a tervalent transition metal cation (yttrium ion). Yttrium ions preferentially bind to inositol polyphosphates, and the sensitivity of the method is greatest for the higher inositol polyphosphates, i.e., the peak area obtained for a specific amount of substance is >50% greater for IP6, compared with 1,4,5-IP3. Moreover, a drastic increase in isomer selectivity is obtained for inositol polyphosphates containing more than three phosphate groups. In this study, the identities of inositol polyphosphates were determined by coelution with standards, and additional structural characterization has not been performed. Thus, the 1,4,5-IP3 peak may also contain other IP3 isomers, e.g., 1,3,4-IP3 and 2,4,5-IP3, whereas all higher inositol polyphosphates are nearly pure (26). Furthermore, it has been demonstrated that tissues can also contain cyclic inositol phosphates (36). The physiological role of these compounds is unclear, because cyclic IP3 is only one tenth to one twentieth as potent as 1,4,5-IP3 in releasing calcium from intracellular stores (37). However, when inositol phosphate levels are measured in cells, these compounds cannot be ig-

nored, because the acid treatment used to terminate tissue incubation results in the hydrolysis of the cyclic bond and thus leads to slightly higher contents of noncyclic phosphates (38). Total tissue content of 1,4,5-IP3, including cyclic IP3 and other IP3 isomers, has been determined with a variety of techniques, e.g., fluorometry or gas chromatography. There are considerable differences in the basal 1,4,5-IP3 content of different tissues, e.g., rat brain, 497 pmol/mg of protein; rat salivary gland, 99 pmol/mg of protein; NG108–15 cells, 11.5 pmol/10^6 cells; NIH 3T3 fibroblasts, 8.1 pmol/10^6 cells; and human fibroblasts, 300 pmol/10^6 cells (for review, see Ref. 38). We found, in rat hearts, a 1,4,5-IP3 concentration of 7.4 ± 0.5 pmol/mg of wet weight under basal conditions and of 13.2 ± 0.6 pmol/mg of wet weight after stimulation with 10 μM phenylephrine. In order to relate these concentrations to milligrams of protein, the data should be multiplied by a factor of 10–15. The relevance of tissue concentration differences, in terms of a second messenger role for 1,4,5-IP3, remains unclear. But one has to consider that the total concentration of 1,4,5-IP3 measured might differ quite markedly from the free concentration in the tissues. Further, it remains unclear how much 1,4,5-IP3 is necessary to obtain a physiological effect. Possibly, a relatively small increase in 1,4,5-IP3 may be capable of producing a significant physiological effect.

In numerous tissues, IP5 and IP6 have been reported to be present at high (millimolar) mass levels, whereas 1,4,5-IP3 was present at micromolar levels (5). The present data show that 1,4,5-IP3 had the highest concentration of all inositol phosphates in the heart. The reason for the discrepancy is not clear.

We conclude that the heart, like other tissues, contains agonist-sensitive and agonist-insensitive inositol polyphosphates. IP5 and IP6 also exist in the heart. Concentration- and time-dependent changes in 1,4,5-IP3 and 1,3,4,5-IP4 suggest that they may be related to initiation and maintenance of the force of contraction. Further characterization of the specific pathways by which the inositol polyphosphates in the heart are metabolized and regulated should provide further insights into the physiological role of these compounds.

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