Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6899–6903, November 1984
Physiological Sciences

Pharmacomechanical coupling in smooth muscle may involve phosphatidylinositol metabolism

(cholinergic contraction/receptor transduction)

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Communicated by John R. Brobeck, July 23, 1984

ABSTRACT **Cholinergic contraction of canine trachealis muscle, a contraction that primarily utilizes membrane potential-independent mechanisms for activating contractile proteins (pharmacomechanical coupling), is associated with a decline in the phosphatidylinositol pool, an increase in the phosphatidic acid and diacylglycerol pools, and an increased incorporation of $^{32}PO_4$ into phosphatidylinositol. We found that these changes occur during development of the contraction and during maintenance of tension and are independent of membrane depolarization or increases in cytosolic Ca^{2+} concentration. These findings suggest that phosphatidylinositol turnover may be part of a receptor transduction process controlling receptor-operated Ca^{2+} channels or other membrane potential-independent mechanisms involved in pharmacomechanical coupling in smooth muscle.**

Smooth muscle, unlike skeletal or cardiac muscle, can be activated to contract by using mechanisms that are independent of membrane depolarization (1, 2). The unknown process by which receptor occupancy by an agonist can trigger, in a membrane potential-independent manner, activation of contractile proteins has been termed "pharmacomechanical coupling" (2). There is scarcely any information about mechanisms involved in such coupling. In some smooth muscles, agonist receptor occupancy can activate both membrane potential-dependent and -independent coupling mechanisms (1). There is evidence for at least two types of plasma membrane Ca^{2+} channels, a fast potential-dependent channel and a slower, less selective, receptor-operated, potential-independent channel (3, 4). That pharmacomechanical coupling may be at least partially dependent on extracellular Ca^{2+} (5, 6) suggests that receptor-operated potential-independent channels may be activated in pharmacomechanical coupling contractions. Devine *et al.* (7) found that different drugs evoked unequal maximal contractions of the rabbit main pulmonary artery and that unequal contractions persisted when the tissue was bathed in Ca^{2+}-free high-potassium depolarizing solution. This suggests that pharmacomechanical coupling in rabbit main pulmonary artery may involve intracellular Ca^{2+} release. At present, details are not available about relative roles of Ca^{2+} release or influx or possible effects of altering cytosolic Ca^{2+}–tension relationships during pharmacomechanical coupling contractions.

Numerous studies in nonmuscle tissues indicate that receptor activation of phosphoinositide turnover [the phosphatidylinositol (PtdIns) cycle] might be involved in the transduction of a "signal" between the receptor and a channel or mechanism that is supplying Ca^{2+} to the cytosol (8–11). In this paper we use the term "PtdIns turnover" to indicate the metabolism of phosphoinositides. Recent data indicate that the polyphosphoinositides, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate, may be in equilibrium with PtdIns, and that the activation of phosphatidylinositol 4,5-bisphosphate diesterase may initiate "turnover" (12–14). The phosphoinositide cycle appears to involve the following metabolites: PtdIns, phosphatidylinositol 4-phosphate, phosphatidylinositol 4,5-bisphosphate, diacylglycerol, phosphatidic acid (PtdOH), cytidine diphosphodiacylglycerol, inositol 1,4,5-trisphosphate, inositol 1,4-bisphosphate, inositol 1-phosphate, and inositol (12–14). The link between PtdIns turnover and Ca^{2+} transport or release from internal stores is unknown. Unsaturated diacylglycerol has been shown to be a specific activator of kinase C, which may phosphorylate regulatory enzymes and proteins (15–17). PtdOH or inositol 1,4,5-trisphosphate may function in Ca^{2+} transport or release (18–20). Other possible relationships between inositol lipids and membrane function have been discussed by Allan (21).

The PtdIns cycle has not been completely studied in any smooth muscle. ^{32}P and [^{3}H]inositol labeling experiments and studies in which PtdIns or PtdOH has been measured chemically indicate that PtdIns turnover occurs with activation by various agonists (22–26), may parallel development of contraction (22–24), and may be independent of both membrane depolarization and increases in cytosolic Ca^{2+} (22–24). To our knowledge, PtdIns turnover has not been previously studied during contraction of smooth muscle activated through pharmacomechanical coupling.

In the present study PtdIns turnover was investigated during a cholinergic muscarinic contraction of canine trachealis muscle. There is previous evidence that this contraction in canine trachealis muscle is dependent on pharmacomechanical coupling mechanisms for activation (5, 6). New techniques for quantitation of lipids have allowed us precisely to measure phospholipid pool sizes in small quantities of smooth muscle and easily to perform serial measurements of the content of major phospholipids and diacylglycerol during tension development and during the tonic phase of this contraction.

MATERIALS AND METHODS

Preparations. Strips were dissected from the tracheae of mongrel dogs as described (27), using great care to remove fascia and fat. In six experiments, 5–10 strips weighing a total of 50–500 mg were placed, free floating, in tubes containing 10 ml of medium composed of 137 mM Na^+, 5.9 mM K^+, 2.5 mM Ca^{2+}, 1.2 mM $Mg2^+$, 134 mM Cl^-, 1.2 mM $H_2PO_4^-$, 20.0 mM HCO_3^-, and 1.5 mM glucose. Solutions containing the tissue were incubated at 37°C and gassed continuously with O_2/CO_2 (97:3, vol/vol), giving a pH of 7.30–7.35. The medium was changed two times at 30-min intervals. The general protocol was to add carbamoylcholine to give 5.5 μM and allow the incubation to proceed for 0, 1, 3, or 5 min. The

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Abbreviations: PtdIns, phosphatidylinositol; PtdOH, phosphatidic acid; PtdSer, phosphatidylserine; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine.

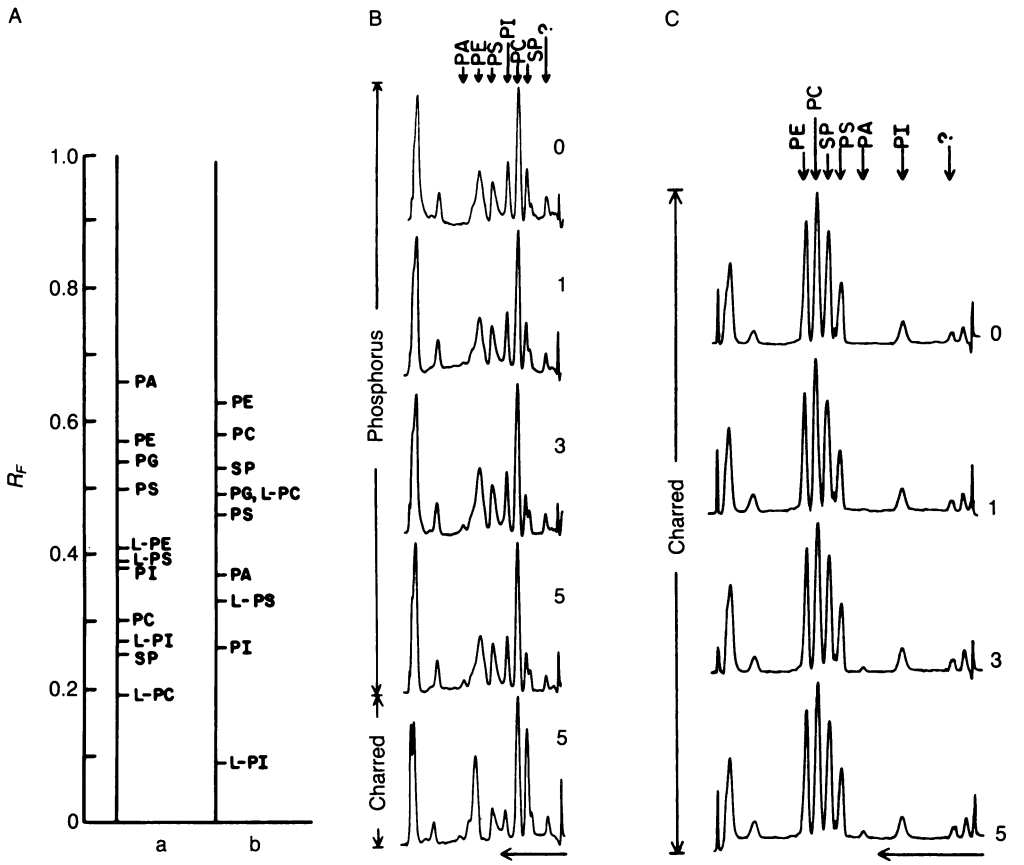

FIG. 1. Thin-layer chromatography of phospholipids. (*A*) R_Fs of phospholipid standards on 20 × 20 cm boric acid-impregnated LK-5 plates. Parts a and b indicate use of mobile phase a and mobile phase b. (*B* and *C*) Data obtained from tissue under control conditions (0 min) and after 1, 3, and 5 min of carbamoylcholine (5.5 μM). Data shown in *B* were obtained with mobile phase a. The samples contained 100 nmol of lipid for phosphorus stain and 50 nmol for charring. Data shown in *C* were obtained with mobile phase b. Samples contained 35 nmol of lipid. Note that lyso-PtdCho (L-PC) appeared between PtdSer (PS) and sphingomyelin (SP) but was observed in only ⅓ of the experiments. PA, PtdOH; PE, PtdEtn; PC, PtdCho; PG, phosphatidylglycerol; PI, PtdIns; and L-, a lysolipid.

solution containing the tissue was rapidly filtered through two layers of cotton gauze [previously washed with chloroform/methanol (2:1, vol/vol)]. The gauze containing the strips was plunged into liquid N_2 and stored under liquid N_2 until further processing.

In four experiments, 20- to 30-mg single strips were mounted in an organ bath that allowed measurement of isometric tension (27). The organ bath was constantly perfused with aerated modified Krebs solution (described above). After a 60-min incubation, the strips were briefly stretched to 0.5 g tension (1 g tension = 9.8 millinewtons) and allowed to relax. The strips were then slowly stretched to 1.3 times slack length. By using tongs cooled in liquid N_2, strips were freeze-clamped under control conditions or after injection of carbamoylcholine. The organ bath was mounted on an apparatus and could be rapidly lowered, allowing freeze-clamping within 1 sec.

Lipid Analysis. The tissue was ground to a fine powder under liquid N_2 and the lipids were extracted (28) and filtered through glass wool. Lipids were analyzed by using thin-layer chromatography and charring densitometry. Methods used for resolving phospholipids and quantitating them are given more completely elsewhere.* Thin-layer chromatography of phospholipids was performed on 20 × 20 cm (15-cm scored lanes) boric acid-impregnated (29) LK-5 plates by using chloroform/ethanol/triethylamine/water (30:34:30:8, vol/vol) (30) (mobile phase a) and chloroform/ethanol/concentrated ammonium hydroxide/water (30:50:9:6, vol/vol) (mobile phase b). Neutral lipids were analyzed on LK-5 plates by successive developments with benzene/diethyl ether/ethyl acetate/acetic acid (80:10:10:0.2, vol/vol) (31) to ¾ of the final solvent front and hexane/diethyl ether (94:6, vol/vol) (32) or with chloroform/methanol (47:3, vol/vol) to 1.5 cm above the origin and hexane/diethyl ether/acetic acid (62/13/0.75, vol/vol) to the top of the plate (33). Samples were spotted and the plate was dried under reduced pressure at 25°C for 20 min prior to development. After development, plates were dried under reduced pressure at 100°C for 20 min, sprayed with 10% $CuSO_4$ in 8% H_3PO_4 (29), and charred by successive incubations at 120°C and 170°C. Some plates were stained for phosphorus by spraying with 12% ammonium molybdate/0.43 M HCl/13% $HClO_4$ and heating at 105°C for 20 min (34, 35). Each plate contained a set of the following quantitated standards: phosphatidylserine (PtdSer), sphingomyelin, phosphatidylcholine (PtdCho), phosphatidylethanolamine, (PtdEtn), PtdIns, and PtdOH, and experimental values are expressed as nmol/100 nmol of these six phospholipids in the samples. The lower limit of charring densitometry is about 0.02 nmol (15 pg) of lipid.

Charring densitometry using $CuSO_4$ in H_3PO_4 was chosen for two reasons. First, this procedure stains both saturated and unsaturated acyl chains equally, whereas $Cu(OAc)_2$ in H_3PO_4 (under the charring conditions used) does not detect saturated acyl chains and gives increased staining with increased numbers of double bonds (36). Second, the background of phosphorus-stained plates varied too much from

*Baron, C.B. & Coburn, R.F. *22nd Eastern Analytic Symposium*, New York, N.Y., Nov. 16–18, 1983, p. 146.

plate to plate for consistent quantitation during densitometry. Densitometry was performed with a fiber optic scanner (Kontes, model 800), equipped with either a 506 ± 45 nm or a 440 ± 150 nm filter for charred plates and a 740 ± 40 nm filter for phosphorus-stained plates, and an integrator (Hewlett Packard, model 3390A). Fig. 1 illustrates the ability of the two mobile phases employed in the thin-layer chromatographic analyses to resolve the phospholipids.

^{32}P Experiments. Free-floating strips were incubated in one tube with $^{32}PO_4$ (150–200 μCi/ml; 1 Ci = 37 GBq) for 30 min and then washed until the radioactivity in the bathing solution became constant. Uptake of ^{32}P varied from 0.1% to 0.5% in the 30-min incubation period. The strips were weighed and placed in multiple tubes, as above, and were further incubated for 60 min prior to addition of carbamoylcholine. Lipids were visualized by spraying with 0.05% rhodamine 6G in 95% (vol/vol) methanol followed by viewing under ultraviolet light. Lipid fractions were scraped into vials and their radioactivities were measured with a liquid scintillation counter and Biofluor.

Materials. Phospholipid standards were from Avanti Biochemicals and Sigma. Neutral lipid standards were from Nu Chek Prep. $H_3{}^{32}PO_4$ and Biofluor were from New England Nuclear. Carbamoylcholine chloride was from Sigma. Linear-K silica gel (LK-5) plates were from Whatman. Rhodamine 6G was from Supelco (Bellefonte, PA). All solvents were glass distilled or nanograde.

RESULTS

Carbamoylcholine-induced isometric contractions showed an initial fast phase over the first 50–70 sec resulting in approximately 80% of maximal tension, followed by a slow phase that reached maximal tension after 4–5 min. Tension was then maintained nearly constant for at least 30 min. Thus, the 1-min samples that were analyzed for lipid composition and radioactivity were taken at the end of the rapid contractile phase, whereas the 3- and 5-min analyses gave information during the slow phase and at the time of maximal tension. Fig. 2 shows mechanical effects of 5.5 μM carbamoylcholine on canine trachealis muscle tension.

Table 1 and Fig. 3 summarize results of the lipid analyses. Phospholipid phosphorus (37) in this tissue was found to be 10.3 ± 0.7 nmol/mg wet wt (n = 24). There were, in both free-floating and isometric experiments, large progressive increases in PtdOH pool size that stoichiometrically paralleled falls in PtdIns during the first 3–5 min. These changes were observed with both mobile phases in charred and phosphorus-stained plates (Fig. 1). There were no significant changes in other phospholipids (Table 1). In a separate free-floating experiment in which duplicate tissue samples were incubated for 20 min, the rise in PtdOH, from 0.02 to 0.80 nmol/100 nmol of phospholipid, and the fall in PtdIns, from 5.04 to 3.96 nmol/100 nmol of phospholipid, suggested that there was no further change in pool size after 5 min. The diacylglycerol pool size increased slightly, but significantly, in the isometric experiments. A similar increase was seen also in the free-floating experiments, but this was not significant. In analyzing tissue from isometric experiments, we utilized an improved method of separating neutral lipids (chloroform/methanol followed by hexane/diethyl ether/acetic acid) that allowed detection of smaller changes in diacylglycerol; thus, these data that showed significant changes in diacylglycerol pool size were more valuable evidence of a change in this lipid. ^{32}P was incorporated into PtdIns during development of carbamoylcholine contractions (Fig. 3*B*). Atropine, 1–10 μM, completely inhibited 5.5 μM carbamoylcholine contractions, ^{32}P incorporation into PtdIns, and increases in PtdOH and falls in PtdIns. There was no increase in ^{32}P incorporation into PtdIns (Fig. 3*B*) or changes in PtdIns or PtdOH during 5-min 29 or 59 mM K^+ contractions. Fig. 4 illustrates effects of varying carbamoylcholine contraction on PtdOH increases.

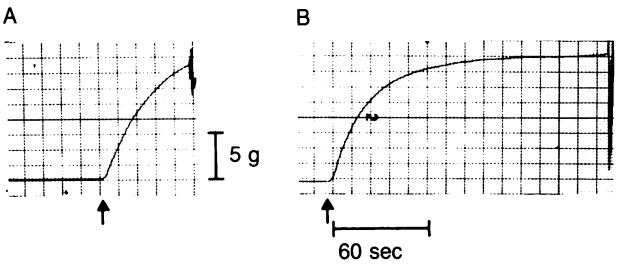

FIG. 2. Isometric carbamoylcholine contractions. Arrows indicate addition of carbamoylcholine. The run shown in *A* was freeze-clamped at 1 min and the run in *B* was freeze-clamped at 3 min.

Table 1. Phospholipid and diacylglycerol composition of carbamoylcholine-stimulated canine trachealis muscle

Lipid	Lipid, nmol/100 nmol of phospholipid			
	Control	1 min	3 min	5 min
		Free floating		
PtdIns	4.52 ± 0.58	4.22 ± 0.72*	4.07 ± 0.47*	3.84 ± 0.71*
PtdOH	0.13 ± 0.13	0.51 ± 0.47*	0.63 ± 0.30*	0.85 ± 0.30*
PtdSer	9.95 ± 1.98	9.63 ± 1.69	9.55 ± 1.66	9.73 ± 1.86
Sphingomyelin	16.4 ± 0.9	16.3 ± 0.9	17.2 ± 0.9	16.6 ± 0.9
PtdCho	42.1 ± 5.7	42.6 ± 4.8	42.8 ± 4.8	42.5 ± 5.0
PtdEtn	26.9 ± 4.9	26.4 ± 5.3	25.5 ± 4.9	26.0 ± 4.8
Diacylglycerol†	0.29 ± 0.16	0.27 ± 0.10	0.39 ± 0.18*	0.32 ± 0.17
		Isometric		
PtdIns	4.03 ± 0.09	3.68 ± 0.15*	3.58 ± 0.27*	3.71 ± 0.15*
PtdOH	0.10 ± 0.08	0.32 ± 0.04*	0.78 ± 0.13*	0.77 ± 0.13*
PtdSer	12.1 ± 1.0	11.5 ± 0.8	11.9 ± 0.8	11.9 ± 0.9
Sphingomyelin	13.9 ± 1.3	13.8 ± 1.7	14.2 ± 1.7	13.9 ± 1.1
PtdCho	36.7 ± 0.5	37.2 ± 0.8	36.2 ± 0.8	36.4 ± 1.0
PtdEtn	33.2 ± 2.5	33.5 ± 2.3	33.5 ± 1.9	33.3 ± 1.4
Diacylglycerol†	0.15 ± 0.05	0.17 ± 0.03	0.24 ± 0.02*	0.23 ± 0.03*

The data were obtained by thin-layer chromatography and charring densitometry. Values are expressed as mean ± SD. For free-floating muscle, n = 6; for isometric, n = 4.
*$P < 0.05$ compared to control (calculated only for PtdIns, PtdOH, and diacylglycerol.
†Assuming the molecular weight of diacylglycerol is that of 1,2-dioleoyl glycerol.

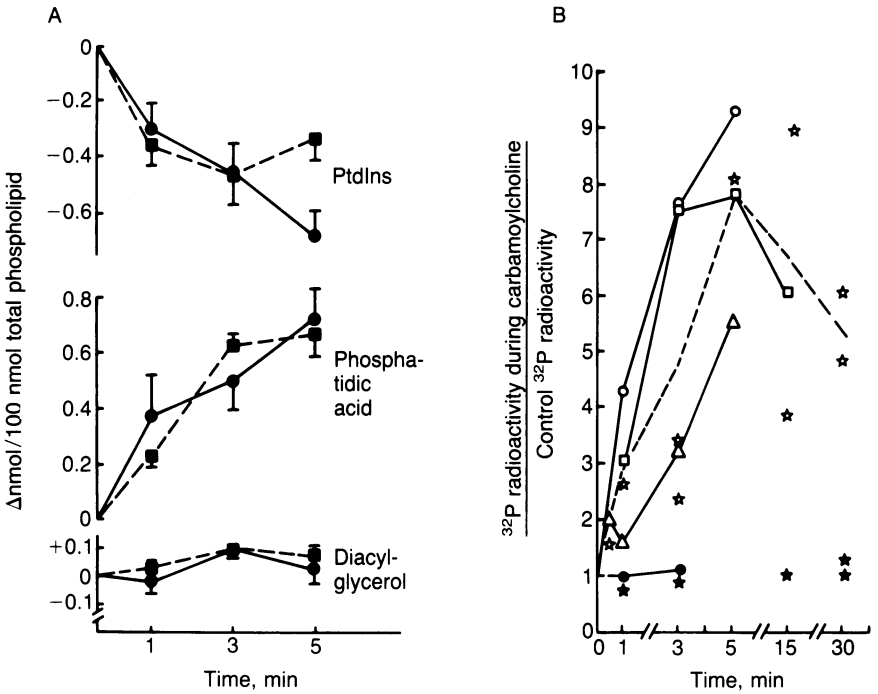

FIG. 3. Changes in PtdIns, PtdOH, and diacylglycerol and ^{32}P incorporation after addition of carbamoylcholine. (*A*) Mean ± SEM of six free-floating experiments (●—●) and four isometric experiments (■---■). Values were obtained by subtracting the value of the control, in individual experiments, from values at 1, 3, and 5 min. (*B*) ^{32}P incorporation into PtdIns during exposure to 5.5 μM carbamoylcholine (open symbols) and during 29–59 mM K^+ contractions (closed symbols). In these experiments 59 mM K^+ contractions had amplitudes similar to those of 5.5 μM carbamoylcholine contractions, and 5.5 μM carbamoylcholine evoked contractions that were 80–90% of maximal carbamoylcholine contractions. Data obtained in the same experiments are connected. Stars indicate that only one measurement was made in a given experiment. Broken line indicates mean values.

DISCUSSION

The major findings in the present study are the decline in PtdIns, an increase in PtdOH and diacylglycerol pool sizes, and incorporation of $^{32}PO_4$ into PtdIns that occurred during development and maintenance of the 5.5 μM carbamoylcholine contraction of canine trachaelis muscle. These findings establish that PtdIns degradation and resynthesis occur during contraction in a smooth muscle that primarily utilizes membrane potential-independent mechanisms for activating contractile proteins.

Previous data have shown that canine trachealis muscle depolarized with 126 mM K^+ contracts on addition of acetylcholine (5). At least 5–10 times more tension development for a given membrane depolarization occurs with acetylcholine compared to that seen with increased extracellular K^+ (5). Membrane potential–acetylcholine concentration plots show that incremental drug concentrations greater than 1 μM cause additional contraction without further membrane depolarization (6). Reversal of 5.5 μM acetylcholine-evoked membrane depolarization by current injection does not reverse the contraction, even though a similar procedure completely reverses K^+ contractions (5). That contractions evoked with high acetylcholine concentrations are resistant to Ca^{2+} entry blockers (5, 6) in this muscle as well as in other airway smooth muscle (38) suggests that membrane potential-dependent Ca^{2+} channels are of small importance in these contractions. Thus, it seems established that at least the tonic phase of 5.5 μM acetylcholine contractions is dependent on pharmacomechanical coupling mechanisms.

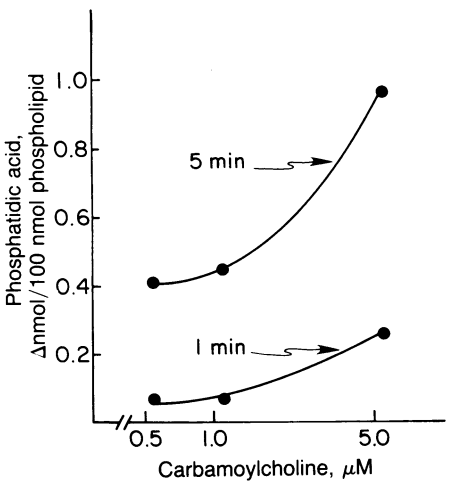

FIG. 4. Effects of varying carbamoylcholine concentration on PtdIns hydrolysis to PtdOH. Lines connect data obtained at 1- and at 5-min drug exposure.

Although cholinergic contractions of canine trachealis muscle may not be mediated entirely by pharmacomechanical coupling mechanisms, the demonstration in this study that PtdIns turnover was not activated during K^+ contractions suggests that PtdIns metabolism is not involved in membrane potential-dependent mechanisms for activation of this muscle. This evidence includes both lack of an increase in ^{32}P incorporation into PtdIns and lack of changes in PtdIns, PtdOH, or diacylglycerol pool sizes.

Considering the previous studies from other tissues discussed above, data obtained in the present study all are consistent with a hypothesis that PtdIns turnover is part of a receptor transduction mechanism in pharmacomechanical coupling smooth muscle contractions. These data include: (*i*) the kinetic changes in PtdIns, PtdOH, and diacylglyercol and in ^{32}P incorporation into PtdIns; (*ii*) the correlation between these changes and development of tension; and (*iii*) the independence of membrane potential and cytosolic Ca^{2+} (deduced from results of K^+ experiments). Because of the lag time for activation due to diffusion of the drug into tissue, we

cannot be certain that lipid changes precede tension changes. However, we can conclude from the finding of a progressive fall in the PtdIns pool, increase in the PtdOH pool, and incorporation of isotope in PtdIns that PtdIns turnover continued during the tonic phase of the carbamoylcholine contraction.

It was possible, using our sensitive techniques of lipid analysis, to study lipid metabolism in single smooth muscle strips held under known mechanical conditions. Comparison of data obtained by using the free-floating and isometric preparations suggests that the amount of stretch or whether the strip contracts isotonically or isometrically may not markedly influence PtdIns turnover.

Our experiments were designed to determine if PtdIns turnover occurs during development and maintenance of a pharmacomechanical coupling contractions and we did not measure, in this series of experiments, changes in polyphosphoinositides or inositol phosphates. Such measurements in smooth muscle would, of course, be of great interest because these metabolites may function as second messengers (19, 20). The 6.5- to 7.7-fold increase in PtdOH pool size (3- to 5-min carbamoylcholine exposures) implies a rapid rise in membrane concentrations of this lipid, which could then elicit effects on membrane proteins, Ca^{2+} transport, or Ca^{2+}–tension relationships. However, the increase in PtdOH pool may reflect, in part, increases in PtdOH in the endoplasmic reticulum as a result of transfer from the plasma membrane. The large increase in PtdOH pool size during the tonic phase of contraction suggests that either those enzymes that use PtdOH as a substrate or the processes involved in transfer of PtdOH from the plasma membrane to the endoplasmic reticulum have become limiting. The rise in diacylglycerol pool (about 1.6 fold) is consistent with a hypothesis that activation of kinase C could have a role in cholinergic muscarinic contractions in the canine trachealis muscle.

In summary, this study provides measurements of PtdIns and PtdOH pool sizes and $^{32}PO_4$ incorporation into PtdIns during a cholinergic muscarinic contraction of canine trachealis muscle, a contraction that appears to be primarily mediated by pharmacomechanical or membrane potential-independent coupling mechanisms. These measurements show that PtdIns degradation and resynthesis occur during a pharmacomechanical-mediated contraction and that changes in PtdIns, PtdOH, and diacylglycerol pool sizes occur during development and maintenance of tension.

We thank Drs. Joseph C. Touchstone and Sidney S. Levin for use of their fiber optic scanner and their valuable assistance, Dr. A. P. Somlyo for his critique of the manuscript, and Maritsa Tzimas for her technical assistance. This work was supported by Grant HL 19737 from the National Heart, Lung, and Blood Institute.

1. Evans, D. H. L., Schild, H. O. & Thesleff, S. (1958) *J. Physiol. (London)* **143,** 474–485.
2. Somlyo, A. V. & Somlyo, A. P. (1968) *J. Pharmacol. Exp. Ther.* **159,** 129–145.
3. Bolton, T. B. (1979) *Physiol. Rev.* 59, 606–718.
4. Smith, R. D. (1983) *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **42,** 201–206.
5. Coburn, R. F. (1979) *Am. J. Physiol.* **236,** C177–C184.
6. Farley, J. M. & Miles, P. R. (1977) *J. Pharmacol. Exp. Ther.* **201,** 199–205.
7. Devine, C. E., Somlyo, A. V. & Somlyo, A. P. (1972) *J. Cell Biol.* **52,** 690–718.
8. Takenawa, T. (1982) *Cell Calcium* **3,** 359–368.
9. Berridge, M. J. (1981) *Mol. Cell. Endocrinol.* **24,** 115–140.
10. Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. & Creba, J. A. (1981) *Philos. Trans. R. Soc. London, Ser. B* **296,** 123–138.
11. Putney, J. W., Jr. (1981) *Life Sci.* **29,** 1183–1194.
12. Berridge, M. J. (1983) *Biochem. J.* **212,** 849–858.
13. Creba, J. A., Downes, C. P., Hawkins, P. T., Brewster, G., Michell, R. H. & Kirk, C. J. (1983) *Biochem. J.* **212,** 733–747.
14. Downes, P. & Michell, R. H. (1982) *Cell Calcium* **3,** 467–502.
15. Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T. & Nishizuka, Y. (1979) *J. Biol. Chem.* **254,** 3692–3695.
16. Kaibuchi, K., Sano, K., Hoshijima, M., Takai, Y. & Nishizuka, Y. (1982) *Cell Calcium* **3,** 323–335.
17. Naka, M., Kishukawa, M., Adelstein, R. S. & Hidaka, H. (1983) *Nature (London)* **306,** 490–492.
18. Serhan, C., Anderson, P., Goodman, E., Dunham, P. & Weissmann, G. (1981) *J. Biol. Chem.* **256,** 2736–2741.
19. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. (1983) *Nature (London)* **306,** 67–68.
20. Thomas, A. P., Marks, J. S., Coll, K. E. & Williamson, J. R. (1983) *J. Biol. Chem.* **258,** 5716–5725.
21. Allan, D. (1982) *Cell Calcium* **3,** 451–465.
22. Egawa, K., Sacktor, B. & Takenawa, T. (1981) *Biochem. J.* **194,** 129–136.
23. Takenawa, T., Masaki, T. & Goto, K. (1983) *J. Biochem.* **93,** 303–306.
24. Salmon, D. M & Honeyman, T. W. (1980) *Nature (London)* **284,** 344–345.
25. Jafferji, S. S. & Michell, R. M. (1976) *Biochem. J.* **160,** 397–399.
26. Aktar, R. A. & Abdel-Latiff, A. A. (1978) *J. Pharmacol. Exp. Ther.* **204,** 655–668.
27. Yamaguchi, T., Hitzig, B. & Coburn, R. F. (1976) *Am. J. Physiol.* **230,** 1737–1743.
28. Christie, W. W. (1973) *Lipid Analysis* (Pergamon, Oxford), p. 39.
29. Fine, J. B. & Sprecher, H. (1982) *J. Lipid Res.* **23,** 660–663.
30. Touchstone, J. C., Levin, S. S., Dobbins, M. F. & Carter, P. J. (1981) *J. High Res. Chromatogr. Chromatogr. Commun.* **4,** 423–424.
31. Storry, J. E. & Tuckley, B. (1966) *Lipids* **2,** 501–502.
32. Freeman, C. P. & West, D. (1966) *J. Lipid Res.* **7,** 324–327.
33. Van der Vusse, G. J., Roemen, T. H. M. & Reneman, R. S. (1980) *Biochim. Biophys. Acta* **617,** 347–352.
34. Wagner, H., Horhammer, L. & Wolff, P. (1961) *Biochem. Z.* **334,** 175–184.
35. Zweig, G. & Sherma, J. eds. (1971) *Handbook of Chromatography*, (Chemical Rubber, Cleveland, OH), No. 235, p. 143.
36. Baron, C. B. & Coburn, R. F. (1984) *J. Liq. Chromatogr.*, in press.
37. Ames, B. N. (1966) *Methods Enzymol.* **8,** 115–118.
38. Eberlin, L. B., Cherniack, A. D. & Deal, E. C., Jr. (1982) *Am. Rev. Resp. Dis. Suppl.* **125,** 225–233.