PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 176, 32–37 (1984)

Attenuation of Psychosocial Stress-Induced Hypertension by Gamma-Linolenic Acid (GLA) Administration in Rats (41838)

D. E. MILLS AND R. WARD

Department of Health Studies, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

Abstract. This study investigated a model of psychosocial stress-induced hypertension in the rat, and examined effects of the prostaglandin E precursor, gamma-linolenic acid (GLA) on the development of hypertension during psychosocial stress. In the first study, male rats were housed four/cage for an acclimation period of 21 days, followed by a 14-day control period. An experimental group ($N = 12$) was then placed in isolation cages for 14 days, then regrouped for a 7-day recovery period. Controls ($N = 12$) remained group-housed. Eight animals per group were sacrificed after the experimental period, and four per group after recovery for organ weight analysis. Mean systolic blood pressure (BP) was similar between groups during the control period (126 ± 2 and 125 ± 2 mm Hg), but increased during isolation, reaching 140 ± 2 mm Hg ($P < 0.001$) by Day 14. During recovery BP returned to control levels. No changes in heart rate, heart weight/body weight or adrenal weight were seen. The second study utilized a protocol similar to that of the experimental group of the first study, minus the recovery period. On Day 1 of the control period 28-day osmotic pumps were implanted ip, releasing olive oil or GLA in olive oil. Four groups of rats ($N = 8$/group) received either (i) olive oil (controls), (ii) 0.018 mg GLA/hr, (iii) 0.040 mg GLA/hr, or (iv) 0.040 mg GLA/hr with no stress. Organ weights were obtained following stress in groups 1–3. Controls developed a sustained elevation in BP within 24 hr of isolation. Animals receiving 0.018 mg GLA/hr developed elevated BP upon isolation, but the BP was less than that of controls on Days 1 ($P < 0.05$) and 14 ($P < 0.001$) of isolation. Animals receiving 0.040 mg GLA/hr demonstrated a greatly attenuated rise in BP vs controls ($P < 0.001$) on all isolation days. GLA in unstressed rats had no effect on BP. Heart rate, heart weight/body weight, and adrenal weight were unchanged in all groups. These data suggest that (i) isolation is a useful tool for investigating reversible psychosocial stress-induced hypertension, and (ii) GLA, while not affecting BP in unstressed animals, produces a dose-dependent attenuation of the BP response to chronic stress.

Prostaglandin E (PGE) has been reported to decrease systemic blood pressure (BP) in both animals (1–4) and man (5–11). However, its clinical usefulness as an antihypertensive agent is limited by its rapid degradation *in vivo* (68–90% inactivated in one pass through the lungs) and side effects, such as dizziness, nausea, and abdominal cramping which result from its hypotensive action (8–11).

It has been suggested that administration of the polyunsaturated fatty acid precursors of the E series prostaglandins, gamma-linolenic acid (GLA) or dihomo-GLA, might elicit decreases in BP while eliminating both the side effects of acute PGE administration, and degradation problems. Studies in rats (12) and rabbits (13) have demonstrated that dietary supplementation with dihomo-GLA increases the dihomo-GLA content of plasma lipids, as well as excretion of the major prostaglandin E-series metabolite, 7 α-hydroxy-5,11-diketo-tetranorprostane-1, 16-dioic acid. Similarly, dihomo-GLA administration in man is associated with elevated platelet dihomo-GLA levels, as well as ability to synthesize PGE (14). These studies suggest that dietary PGE precursors can be used to increase endogenous prostaglandin E_1 (PGE1) and prostaglandin E_2 (PGE2) biosynthesis.

Preliminary studies have also examined the effects of GLA supplementation on BP regulation. Oil of the evening primrose (Efamol), a rich source of GLA and linoleic acid (2-series prostaglandin precursor) has been found to decrease BP in genetically hypertensive rats (15) and in women with pregnancy-induced hypertension (16). These findings suggest that GLA may be useful in controlling pathophysiologic elevations in BP via increasing PGE1 and/or PGE2 synthesis. However, it is not known whether or not PGE or its precursors are effective in modifying reversible

0037-9727/84 $1.50

elevations in BP, such as those which occur during stress, which are believed to contribute, in some instances, to the development of essential hypertension (17–19).

The present study sought to evaluate the effects of administration of the PGE1 and PGE2 precursor, GLA, on psychological stress-induced hypertension in rats. The model for psychological stress used was that of social isolation following group housing, which has been reported to produce a reversible hypertension in Wistar rats (20–22).

Materials and Methods. *Experimental animals.* Fifty-six adult male Sprague–Dawley rats weighing 125–150 g were purchased from Charles River, St. Constant, Quebec. Animals were maintained in a temperature (21 + 1°C)- and light-cycle-controlled room (lights on 0600–2000 hr) throughout the course of the study and were given rat chow and tap water *ad lib.* In addition, all animals were housed in groups (four/cage) for a 3-week acclimation period prior to the beginning of the study.

Agents administered. All exogenous agents used in the study were administered by means of 28 day, constant flow (2.29 μl/hr) osmotic minipumps (Alzet 2ML4) implanted ip under fluothane anesthesia on Day 1 of the study. Gas chromatograph analysis of the GLA (Sigma) demonstrated purity > 99%. The olive oil (OL) carrier for the GLA was shown by gas chromatograph to contain 0.4% alpha-linolenic acid and 9.6% linoleic acid.

Experiment 1. The effects of isolation on BP, heart rate (HR), and organ weight.

Following acclimation all animals remained housed four/cage for a 14-day control period. On Day 15, 12 animals (experimental) were removed from group cages and placed in individual (isolation) cages for a 14-day experimental period. During this time the control animals ($N = 12$) remained housed four/cage. Following the experimental period, isolated animals were regrouped for a 7-day recovery period, while control animals remained in original groups. During the study BP, HR, and body wt were determined on Days 7, 14, 16, 21, 28, and 35. The tail cuff method (23, 24), utilizing a doppler flow transducer was used to measure BP and HR on the conscious rats, which were heated in a 34°C incubator for 10 min prior to measurement. At the end of the experimental period (Day 28), 8 animals from each treatment group were sacrificed, and the heart and adrenal glands were removed and weighed after cleaning and rinsing in physiological saline. Similarly, at the end of the recovery period the remaining 4 animals per group were sacrificed for organ weight determination.

Experiment 2. The effects of GLA on the BP and HR responses to psychosocial stress.

Animals in this experiment underwent a similar protocol as the experimental (stressed) group in Experiment 1, with the following exceptions. Following acclimation, animals were divided into four treatment groups and had 28-day, osmotic minipumps implanted. Pumps released either (i) OL ($N = 8$), (ii) 0.018 mg GLA in OL/hr ($N = 8$), (iii) 0.040 mg GLA in OL/hr ($N = 8$), or (iv) 0.040 mg GLA in OL/hr ($N = 6$) with no stress exposure. Following a 14-day control period (four/cage) all animals, except those in group 4, were isolated for a 14-day experimental period. At the end of the experimental period (Day 28) groups 1–3 were sacrificed for organ weight determination, as previously described. Measurements of BP, HR, and body wt were made on Days 7, 14, 16, 21, and 28. The availability of reagents limited group 4 to six animals, but housing four/cage was permitted by the addition of two nonexperimental animals to one of the cages.

Data analysis. Analysis of BP, HR, and body wt were performed using a 2-way ANOVA on repeated measures. Organ weight data were analyzed using a 2-way ANOVA. Where a significance of $P < 0.05$ was achieved, a planned comparison test (25) was performed to determine which subgroups differed from the others.

Results. *Experiment 1.* The BP response to stress is shown in Fig. 1. Although there were no differences in BP between groups during the control period, isolation produced a significant increase in BP, from 125 ± 2 mm Hg ($\bar{x}$ ± SE) to 137 ± 2 mm Hg ($P < 0.05$ vs grouped), within 24 hr. The BP of stressed animals continued to rise on Days 21 and 28, reaching 140 ± 2 mm Hg ($P < 0.001$ vs grouped), while that of control animals did not change significantly from control period values. Following 7 days of recovery the BP of experimental animals decreased to control period levels (125 ± 2 mm Hg), which were

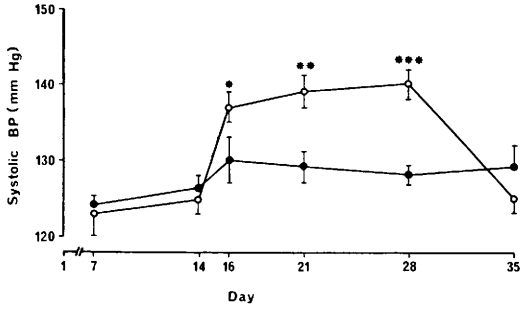

FIG. 1. Systolic blood pressure (BP) of adult male rats group-housed for 5 weeks (●) and of rats placed in isolation for 2 weeks (○) following 2 weeks in group housing. *$P < 0.05$ vs group-housed; **$P < 0.01$ vs group-housed; ***$P < 0.001$ vs group-housed.

not significantly different from control group values. There were no significant changes in HR during the study in either group (data not shown) in comparison to Day 7 means of 308 ± 8 bpm for controls and 347 ± 12 bpm for experimental groups. Similarly, there were no differences in body weight gain by either group during the study (data not shown). Heart and adrenal gland weight data are shown in Table I. On Day 28, mean heart weight of the stressed rats was significantly greater than that of the control rats ($P < 0.001$). However, this difference did not appear in the heart weight/BW ratio. There were no differences in either adrenal weight or adrenal weight/body wt ratio between groups on Day 28. On Day 35 there were no differences in heart or adrenal weights or weight ratios between groups of animals.

Experiment 2. The BP response to GLA treatment during stress is illustrated in Fig. 2. Olive oil-treated rats (controls) demonstrated a significant increase in BP from 128 ± 3 to 146 ± 2 mm Hg ($P < 0.001$) within 24 hr of isolation, which leveled off at 142 ± 2 mm Hg by Day 28. Administration of GLA at a rate of 0.018 mg/hr (1.1 mg/kg/day) had no effect on baseline BP, which was similar to that of OL-treated animals, but slightly attenuated the BP rise 24 hr after isolation (142 ± 2 mm Hg) on Day 16 ($P < 0.05$ vs OL). Furthermore, BP in this group failed to plateau during the isolation period, falling off on Day 28 to 132 ± 2 mm Hg ($P < 0.001$ vs OL). Administration of GLA at a rate of 0.040 mg/hr (2.4 mg/kg/day) also had no effect on baseline BP, but significantly attenuated the isolation-induced BP rise on Days 16 (133 ± 1 mm Hg, $P < 0.001$ vs OL), 21 (132 ± 1 mm Hg, $P < 0.001$ vs OL), and 28 (131 ± 2 mm Hg, $P < 0.001$ vs OL). The administration of 0.040 mg GLA/hr to unstressed rats had no

TABLE I. MEAN BODY (BW), HEART, AND ADRENAL WEIGHTS IN CONTROL AND SOCIALLY ISOLATED RATS FOLLOWING THE EXPERIMENTAL (DAY 28) AND RECOVERY (DAY 35) PERIODS (EXPERIMENT 1), AND IN SOCIALLY STRESSED RATS RECEIVING OLIVE OIL (OL) OR GAMMA-LINOLENIC ACID (GLA) BY OSMOTIC MINIPUMP (EXPERIMENT 2)

	Heart wt (g)	Heart wt/ 100 g body wt (g)	Adrenal wt (g)	Adrenal wt/ 100 g body wt (g)	Body wt (g)
Experiment 1					
Day 28					
Control ($N = 8$)	1.129 ± 0.048	0.268 ± 0.010	0.058 ± 0.003	0.015 ± 0.001	409.6 ± 11.2
Isolated ($N = 8$)	1.319 ± 0.043***	0.307 ± 0.009	0.058 ± 0.002	0.014 ± 0.001	433.4 ± 11.7
Day 35					
Control ($N = 4$)	1.028 ± 0.050	0.241 ± 0.012	0.063 ± 0.005	0.015 ± 0.001	428.7 ± 3.5
Isolated ($N = 4$)	1.098 ± 0.068	0.253 ± 0.006	0.063 ± 0.005	0.015 ± 0.001	435.2 ± 15.9
Experiment 2					
Day 28					
OL ($N = 8$)	1.186 ± 0.052	0.288 ± 0.006	0.060 ± 0.002	0.015 ± 0.001	410.5 ± 13.0
0.018 mg GLA/hr ($N = 8$)	1.175 ± 0.041	0.284 ± 0.011	0.060 ± 0.003	0.015 ± 0.001	413.9 ± 7.5
0.040 mg GLA/hr ($N = 8$)	1.034 ± 0.035*	0.295 ± 0.006	0.062 ± 0.002	0.019 ± 0.007	350.0 ± 5.9***

Note. Values are means ± SE.
* $P < 0.05$ vs controls.
*** $P < 0.001$ vs controls.

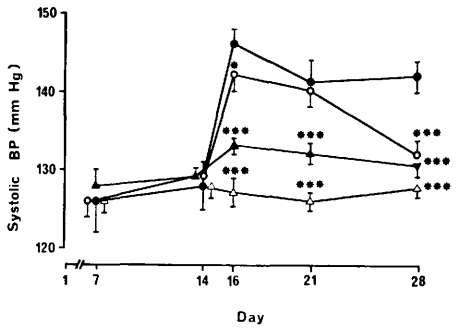

FIG. 2. Systolic BP before and during social isolation in adult male rats receiving olive oil (●, $N = 8$) or GLA at calculated rates of 0.018 mg/hr (○, $N = 8$) and 0.040 mg/hr (▲, $N = 8$) via osmotic minipump implanted ip on Day 1. An additional group receiving 0.040 mg GLA/hr (△, $N = 6$) was not placed in isolation. (Means + SE; $*P < 0.05$ vs olive oil group; $***P < 0.001$ vs olive oil group.

effect on BP over the 28-day treatment period. No significant changes in HR or BW gain were seen in any of the treatment groups over the course of the study, nor were there significant differences in control period HR between groups (data not shown). Similarly, there were no differences in heart weight, adrenal weight, heart weight ratio, or adrenal weight ratio between groups on Day 28 (Table I).

Discussion. In the present study, isolation of group acclimated, intact, male Sprague–Dawley rats for 14 days produced a persistent elevation in BP of 15–20 mm Hg. This hypertension was unaccompanied by an increase in HR over the same period, and was reversed following the cessation of isolation. Similar studies on male Wistar rats undergoing a 15-day isolation period have reported BP increases of 20–25 mm Hg., accompanied by initial elevations in HR which disappeared after 5 days of isolation (20). The variation in response to this psychosocial stress may be related to strain differences in stress sensitivity, or differences in experimental protocol which would cause isolation to be a more severe stressor in the earlier studies.

No changes in BW, heart weight/body weight, or adrenal weight/body weight were observed as a result of isolation in the present study. Similarly, a previous study using social interaction as a stressor has reported no change in body weight gain due to the stress (26), and BP elevations following 12 weeks of electric shock were found to have no effect on heart weight/body weight in normotensive rats (27). However, increases in relative heart weight and adrenal weight have been observed following long-term (months) isolation stress (28), and after electric shock stress (27), respectively. The absence of cardiac and adrenal hypertrophy in the present study may be due to either the shorter duration or different nature of the stressor.

The fact that isolation stress did not produce irreversible hypertension in any studies using normotensive animals (20–22) suggests that either a longer duration of stress than was used is required to produce irreversible changes in BP regulation, or that there are other, possibly genetic, factors that predispose an individual to develop irreversible hypertension from reversible stress-induced hypertension. This is supported by the observation that certain borderline hypertensive rats, related to the genetically hypertensive rat (SHR), develop irreversible hypertension following 12 weeks of electric shock (29).

Another finding of the present experiments is that chronic administration of the PGE precursor, GLA, attenuates the stress-induced BP rise, while not affecting basal (unstressed) BP levels. This effect of GLA on BP during stress occurs in the absence of significant changes in HR, suggesting that GLA is not mediating its effect through an alteration in sympathetic activity. However, the observation that an effect is seen only during presumed heightened sympathetic activity (30) suggests that GLA may be influencing the response to sympathetic stimulation. While there is ample evidence that PGE is able to modify the peripheral vascular response to catecholamines, there is disagreement as to whether the influence is that of potentiation, as demonstrated in certain isolated preparations (31–33, 36), or inhibition, as shown *in vivo* (34, 35). It has also been suggested that at low doses PGE potentiates catecholamine-induced vasoconstriction, and at high doses inhibits it (37).

A possible explanation of why GLA exerts a depressor effect on BP only during stress is that GLA administration might increase dihomo-GLA and/or arachidonic acid stores in the basal condition, so that under conditions of stimulated PGE biosynthesis, there is a greater production of PGE. An increase in

sympathetic activity during stress might cause an increase in PGE1 or PGE2 production which would be augmented by elevated dihomo-GLA and/or arachidonic acid stores resulting from GLA treatment.

A second possibility is that GLA leads to increased PGE levels in the unstressed animal, but that this PGE only exerts its vasodepressor effects when in the presence of elevated catecholamine levels, as during stress. This explanation is unlikely, since PGE administration exerts potent hypotensive activity even under basal conditions (1–7).

The fact that GLA treatment attenuates stress-induced increases in BP, while having no effect on BP in unstressed animals, makes it an attractive prospect for use as an antihypertensive agent, particularly in cases of labile hypertension, such as can occur during periods of stress or other situations involving sympathetic stimulation.

This work was supported by a grant from the Ontario Heart Foundation. The authors thank J. Ward for conducting gas chromatographic analyses of agents used in the study, H. Kong for his technical assistance, and S. Hurlburt for her patient secretarial assistance.

1. Somova IL, Dochev DH. Studies on the mechanism of the diuretic action of prostaglandin E_1. Dokl Bolg Akad Nauk **26**:1277–1282, 1973.
2. Tyler T, Leffler C, Wallis R, Cassin S. The effects of prostaglandins of the E-series on pulmonary and systemic circulations of newborn goats during normoxia and hypoxia. Prostaglandins **10**:963–970, 1975.
3. McQueen D, Bell K. The effects of prostaglandin E_1 and sodium meclofenamate on blood pressure in renal hypertensive rats. Eur J Pharmacol **37**:223–235, 1976.
4. Cassin S, Tyler T, Leffler C, Wallis R. Pulmonary and systemic vascular responses of perinatal goats to prostaglandins E_1 and E_2. Amer J Physiol **236**:H838–H832, 1979.
5. Pipkin FB, O'Brien PMS, Sant-Cassia LJ. The effect of prostaglandin E_1 on the pressor response to angiotensin II in second trimester human pregnancy and in the non-pregnant subject. Clin Exp Hypertens **B1**:493–504, 1982.
6. Bergström S, Carlson LA, Ekelund LG, Oro L. Effects of prostaglandin E_1 on blood pressure, heart rate, and concentrations of free fatty acids of plasma in man. Proc Soc Exp Biol Med **118**:110–112, 1965.
7. Carlson LA, Eriksson I. Femoral artery infusion of prostaglandin E_1 in severe peripheral vascular disease. Lancet **1**:155–156, 1973.
8. Bergström S, Cuner H, von Euler US, Pernow V, Sjovall J. Observations on the effects of infusion of prostaglandin E in man. Acta Physiol Scand **45**:145–151, 1959.
9. Bergström S, Carlson LA, Ekelund LG, Oro L. Cardiovascular and metabolic response to infusions of prostaglandin E_1 and to simultaneous infusions of noradrenaline and prostaglandin E_1 in man. Acta Physiol Scand **69**:332–339, 1965.
10. Carlson LA, Ekelund LG, Oro L. Clinical and metabolic effects of different doses of prostaglandin E_1 in man. Acta Med Scand **183**:423–430, 1968.
11. Carlson LA, Ekelund LG, Orö L. Circulatory and respiratory effects of different doses of prostaglandin E_1 in man. Acta Physiol Scand **75**:161–169, 1969.
12. Willis AL, Comaik K, Kuhn DC, Paulsrud J. Dihomo-gamma linolenate suppresses platelet aggregation when administered *in vitro* or *in vivo*. Prostaglandins **8**:509–519, 1974.
13. Oelz O, Seyberth HW, Knapp HR, Sweatman BJ, Oates JA. Effects of feeding ethyl-dihomo-γ-linolenate on prostaglandin synthesis and platelet aggregation in the rabbit. Biochim Biophys Acta **431**:268–277, 1976.
14. Kernoff BA, Willis AL, Stone KJ, Davies JA, McNicoll GP. Antithrombotic potential of dihomo-γ-linolenic acid in man. Brit Med J **2**:1441–1444, 1977.
15. Hoffman P, Förster W. Effects of sunflowerseed oil, linseed oil, evening primrose oil, and hydrogenated palm kernel oil on hypertension development in spontaneously hypertensive rats. Prostaglandins Med **11**:43–44, 1983.
16. Moodley J, Norman R. Preliminary Studies on efamol in pregnancy-induced hypertension. Prostaglandins Med, in press.
17. Dembroski TM, McDougall JM, Shields JL. Physiologic reactions to social challenge in persons evidencing Type A coronary-prone behavior pattern. J Hum Stress **3**:2–9, 1977.
18. Levi L, Kagan A. Psychosocially-induced stress and disease—problems, research strategies and results. In: Selye H, ed. Selye's Guide to Stress Research. New York, Van Nostrand Reinhold, Vol 1:p118, 1980.
19. Martin RL. Hypertension and Stress. Sem Fam Med **2**:25–34, 1981.
20. Gardiner SM, Bennett T. The effects of short term isolation on systolic blood pressure and heart rate in rats. Med Biol **55**:325–329, 1977.
21. Gardiner SM. The effects of social contact on hypertension induced by short term isolation in the rat. J Physiol **269**:62–63P, 1977.
22. Gardiner SM, Milmer KE, Bennett T. Effect of adrenalectomy on the development of isolation-induced hypertension in rats. Clin Sci **61**:511–519, 1981.
23. Rowberg A, Franklin C, van Citters RL. Nontraumatic method for measurement of blood pressure in animals with tails. J Appl Physiol **27**:301–302, 1969.
24. Buñag RD. Validation in awake rats of a tail-cuff method for measuring systolic pressure. J Appl Physiol **34**:279–282, 1973.

25. Keppel G. Design and Analysis: A Researcher's Handbook. New Jersey, Prentice-Hall, p85, 1973.
26. Henry JP, Stephens PM, Santisteban GA. A model of psychosocial hypertension showing reversibility and progression of cardiovascular complications. Circ Res **36**:156–164, 1975.
27. Lauter JE, Barker GF, Hubbard JW, Schaub RG. Effects of stress on blood pressure and cardiac pathology in rats with borderline hypertension. Hypertension **3**:496–505, 1981.
28. Baer H. Long-term isolation stress and its effects on drug response in rodents. Lab Anim Sci **21**:341–349, 1971.
29. Lawler, JE, Barker GF, Hubbard JW, Schaub RG. Pathophysiological changes associated with stress-induced hypertension in the borderline hypertensive rat. Clin Sci **59**:3075–3105, 1980.
30. Moore KE. Studies with chronically isolated rats: Tissue levels and urinary excretion of catecholamines and plasma levels of corticosterone. Canad J Physiol Pharmacol **46**:553–558, 1968.
31. Greenberg S, Long JP. Enhancement of vascular smooth muscle responses to vasoactive stimuli by prostaglandin E_1 and E_2. Arch Int Pharmacodyn Ther **206**:94–104, 1973.
32. Horrobin DF, Manku MS, Karmali R, Nassar BA, Davies PA. Aspirin, indomethacin, catecholamine, and prostaglandin interactions on rat arterioles and rabbit hearts. Nature (London) **250**:425–426, 1974.
33. Coupar, IM, McLennan PL. The influence of prostaglandins on noradrenaline-induced vasoconstriction in isolated perfused mesenteric blood vessels of the rat. Brit J Pharmacol **62**:51–59, 1978.
34. Weiner R, Kaley G. Influence of prostaglandin E_1 on the terminal vascular bed. Amer J Physiol **217**:563–566, 1969.
35. Kadowitz PJ. Effects of prostaglandins E_1, E_2 and A_2 on vascular resistance and responses to noradrenaline, nerve stimulation, and angiotensin in dog hindlimb. Brit J Pharmacol **46**:495–500, 1972.
36. Malik KU, Ryan P, McGiff JC. Modification by prostaglandins E_1 and E_2, indomethacin, and arachidonic acid of the vasoconstrictor responses of the isolated perfused rabbit and rat mesenteric arteries to adrenergic stimuli. Circ Res **39**:163–168, 1976.
37. Manku MS, Horrobin DF, Karmazyn M, Cunnane SC. Prolactin and zinc effects on rat vascular reactivity: Possible relationships to dihomo-γ-linolenic acid and to prostaglandin synthesis. Endocrinology **104**:774–779, 1979.

Received September 15, 1983. P.S.E.B.M. 1984, Vol. 176.
Accepted January 25, 1984.