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Effect of Prostaglandin F_{2a} (PGF_{2a}) on Venous Contractility and ^{45}Ca Uptake[1] (37752)

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It has been postulated that the ability of any prostaglandin to contract smooth muscle was due to an interaction of prostaglandin with the cell membrane while prostaglandin-mediated potentiation of the contractile responses of smooth muscle to other agonists was dependent on an intracellular action of prostaglandins on the excitation–contraction coupling mechanism (1–2). It was also proposed that prostaglandin-mediated facilitation of vascular reactivity was mediated by removal of calcium from binding sites which normally bind calcium ion (3–4). This results in a membrane which acts as if it is partially depolarized and more permeable to calcium ion. These concepts were supported by the experiments which demonstrated that prostaglandins lower the threshold at which smooth muscle preparations respond to nerve stimulation (1–4).

The following studies were performed to test the postulate that PGF_{2a} interacts with calcium metabolism of vascular smooth muscle. The results demonstrate that PGF_{2a} increases the rate of ^{45}Ca uptake and efflux as well as enhancing norepinephrine-stimulated ^{45}Ca uptake.

Methods and Materials. Mongrel dogs of either sex (8–15 kg) were anesthetized with sodium pentobarbital (35 mg/kg, iv). Dorsal metatarsal branches of the saphenous vein were removed, cut into helical strips and prepared for superfusion as previously described (5). Each vascular strip was mounted at the optimal resting tension. The preparation was allowed to equilibrate 3 hr prior to experimentation. Isometric contractions were measured with a Grass Ft.03 force-displacement transducer and recorded on a Grass Model 5 dynograph. The physiologic salt solution (PSS), maintained at 37.5°, aerated with 95% O_2 and 5% CO_2 (pH 7.4), contained (millimolar concentration): sodium chloride (113), potassium chloride (3.1), sodium bicarbonate (24.8), calcium chloride (1.53), dextrose (10), magnesium chloride (2.1), monopotassium dihydrogen phosphate (1.1), and calcium disodium ethylenediaminetetraacetate (0.026). The flow rate was 3 to 4 ml/min. The calcium concentration utilized in these studies was determined by measurement of plasma calcium concentration and subtraction of the plasma protein bound fraction ($\cong$ 45%).

Contractility studies. Partial dose–response curves were obtained for norepinephrine (0.6–9.6 nmoles) and $BaCl_2$ (24 to 96 μmoles) prior to and during superfusion with PGF_{2a}. Each vascular strip was challenged with the 3 doses of each agonist. These doses of agonist were used approximately 20, 50 and 80% of the maximal contractile response of the tissue for the agonist. Each tissue was chal-

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lenged with increasing doses of norepinephrine every 15 min. Twenty minutes elapsed between each succeeding dose of $BaCl_2$. This procedure resulted in a return to base line tension after each dose of agonist prior to obtaining a response to succeeding doses of agonist. Responses were independent of the order of agonist administration.

Radiocalcium studies. Calcium efflux. Helical strips of canine vascular smooth muscle were mounted on Pyrex rods and incubated for 3 hr in PSS and 1 hr in PSS containing radiocalcium (10 μCi/ml). At the end of this time, the vascular strips, while still on the Pyrex frame, were removed from the labeled PSS, gently blotted with strips of filter paper, and placed into tubes containing tracer-free PSS at 37°. The strips were transferred at variable intervals to test tubes containing fresh PSS for 4 consecutive hours, removed from their frames, blotted, weighed and placed overnight in 5.0 ml of Na_2 EDTA solution (10 m*M*) in 50% (v/v) ethanol (6). The radioactivity of the muscle extract and each wash-out vial was determined with scintillation spectrometry.

Calcium uptake. Dorsal metatarsal veins were equilibrated under tension as described above for 3 hr and then in ^{45}Ca containing PSS (10 μCi/ml/1.53 μmole ^{40}Ca) for varying time periods in the presence and absence of $PGF_{2\alpha}$. Strips were then weighed and placed in PSS containing (m*M*); NaCl (139); KCl (4.1), $MgCl_2$ (2.1) dextrose (10). Tris buffer (20) and lanthanum chloride (10) for 10 min. Lanthanum is believed to inhibit the loss of calcium ion from within the cell while displacing the membrane bound and extracellular fraction of calcium ion. Veins were then extracted as described above and an aliquot of the extract was counted with liquid scintillation spectroscopy with a Packard Tricarb liquid scintillation counter. The effect of $PGF_{2\alpha}$ on norepinephrine-stimulated ^{45}Ca uptake was evaluated by incubating the norepinephrine, $PGF_{2\alpha}$ or both with venous smooth muscle as described above.

Drugs and chemicals. *l*-Norepinephrine bitartrate (Levophed, Sterling-Winthrop Research Institute, New York, NY), barium chloride (Baker Chemical Co., Phillipsburg, NJ). The supply of prostaglandins used in these experiments was generously donated by Dr. J. R. Weeks of the Upjohn Co., Kalamazoo, MI.

Statistical evaluation of data. Dose–effect data were analyzed by analysis of variance for a partially nested, randomized complete block factorial design. Whenever the factorial analysis resulted in a significant interaction term the means were analyzed for significance by the Duncan's new multiple range test or by Tukey's procedure (7). Data from studies designed to evaluate the effect of drugs on radiocalcium uptake was analyzed with Student's group *t* test (7). A *P* value of 0.05 or less ($P < 0.05$) was chosen for statistical significance.

Results. Contractility studies. The responses of each venous strip to norepinephrine and $BaCl_2$ were consistent and reproducible throughout the experimental procedure. The maximal contractile response to each vascular strip to $BaCl_2$ was significantly less ($P < 0.05$) than the responses to norepinephrine (Fig. 1), a phenomena described by many investigators (8–12). Prostaglandin $F_{2\alpha}$ did not affect venous smooth muscle tone ($P > 0.05$).

The effect of $PGF_{2\alpha}$ on the contractile responses to dorsal metatarsal branches of saphenous veins to $BaCl_2$ and norepinephrine is presented in Fig. 2. $PGF_{2\alpha}$ (2.1×10^{-8} *M*) did not alter the responses of this preparation to any of the agonists. The contractile responses to $BaCl_2$ were enhanced greatly with the higher concentrations of $PGF_{2\alpha}$ which only had a relatively small effect on the contractile response to norepinephrine (Fig. 2).

Effect of $PGF_{2\alpha}$ and norepinephrine on calcium uptake into venous smooth muscle. Figure 3 summarizes the uptake of radiocalcium during the various incubation times in radiocalcium. Approximately 95 to 99% of the radiocalcium was exchanged at the end of a 3-hr incubation period. These values are in agreement with those of other investigators (13–15). The extraction procedure removed essentially all of the radioisotope from the tissue as described previously (6).

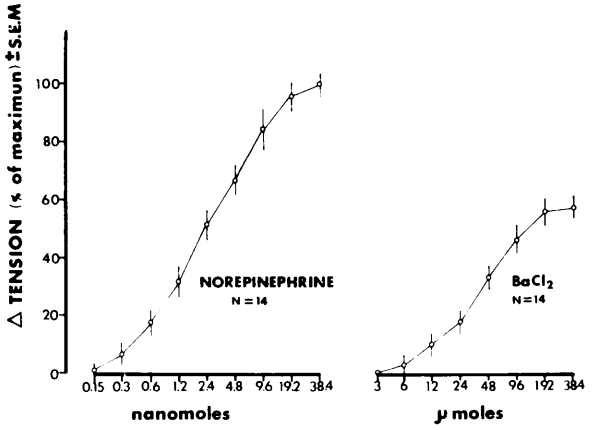

FIG. 1. Contractile responses of superfused helical strips of canine dorsal metatarsal veins to norepinephrine and barium chloride. The ordinate represents the response (Δ tension) to these agonists. The abscissa represents the dose of agonist. *N* represents the number of vascular strips used in the calculation of the mean value. Each vascular strip was obtained from a different dog.

Prostaglandin $F_{2\alpha}$ increased ($P < 0.05$) the uptake of ^{45}Ca into dorsal metatarsal veins. The total uptake did not change. Norepinephrine enhanced the rate and total uptake of ^{45}Ca into dorsal metatarsal veins. $PGF_{2\alpha}$ enhanced norepinephrine-stimulated ^{45}Ca uptake into dorsal metatarsal veins (Fig. 3).

Calcium efflux was also increased when $PGF_{2\alpha}$ was added to the desaturation solution during the slow component of calcium efflux.

Discussion. Barium chloride is believed to initiate contractile responses by entering the smooth muscle cell, activating the contractile proteins and/or releasing calcium from bind-

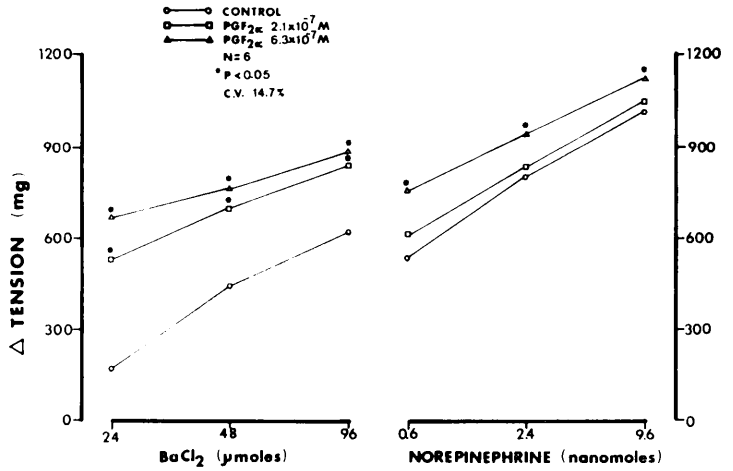

FIG. 2. Effect of $PGF_{2\alpha}$ on contractile responses of superfused helical strips of dorsal metatarsal veins to norepinephrine and barium chloride. The ordinate represents the change in base line tension, (mg) observed after the administration of each of the agonists. The abscissa represents the dose of agonist in micromoles or nanomoles. Closed symbols indicate the control responses. The open symbols represent the responses of the agonist 30 min after the addition of $PGF_{2\alpha}$ to the superfusate. Each point represents the mean of *N* preparations. (*) The responses to a given agonist during superfusion with $PGF_{2\alpha}$ significantly differs ($P < 0.05$) from control values. CV is the coefficient of variation.

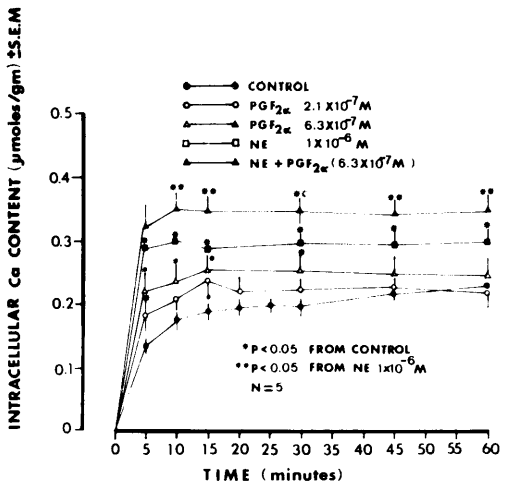

FIG. 3. Effect of $PGF_{2\alpha}$ and norepinephrine on the uptake of radiocalcium into canine dorsal metatarsal venous muscle. The ordinate represents the intracellular ^{45}Ca content (normalized for the specific activity of the isotope and the weight of the vein) in the presence and absence of drug. The abscissa represents the time of incubation. The *N* value represents the number of vascular strips used for calculation of the mean. Each vascular strip was obtained from a different dog. The calcium content of the vascular strips in the presence of drug was compared to its corresponding control with Student's group *t* test (16). (*) Mean values significantly differ ($P < 0.05$) from control uptake obtained in the absence of $PGF_{2\alpha}$.

ing sites within the cell (9–12). Current evidence supports the concept that the contractile response of vascular smooth muscle to catecholamines is mediated by enhanced membrane permeability to calcium ion as well as release of calcium from intracellular binding sites (8–12).

Since $PGF_{2\alpha}$ enhanced the contractile responses of vascular smooth muscle to each of the agonists as well as facilitating norepinephrine-induced ^{45}Ca uptake the results are compatible with the conclusion that high concentrations of $PGF_{2\alpha}$ act at the smooth muscle membrane to enhance membrane permeability to calcium ion. These results support the postulate that facilitation of vascular reactivity by $PGF_{2\alpha}$ appears to be mediated by $PGF_{2\alpha}$ interacting with the cell to alter calcium binding and permeability (1–4).

In addition to its enhancement of norepinephrine-induced ^{45}Ca uptake $PGF_{2\alpha}$ may act within the cell to alter the binding or uptake or radiocalcium to intracellular organelles such as mitochondria, sarcoplasmic reticulum the inner side of the cell membrane of other cell inclusions. This could explain $PGF_{2\alpha}$-induced enhancement of calcium 45 efflux (13–15) (Fig. 4). Alternatively, since the increased isotope in the vein reflects an increased rate of calcium exchange rather than an increase in the actual quantity of calcium entering the cell (Fig. 3), the increased rate of calcium 45 efflux may merely reflect an increased exchange of labeled for nonlabeled calcium ion.

In conclusion, the results of the present experiments demonstrate that $PGF_{2\alpha}$ enhances the responses of venous smooth muscle to barium chloride and norepinephrine. The rate of venous ^{45}Ca uptake is enhanced by $PGF_{2\alpha}$.

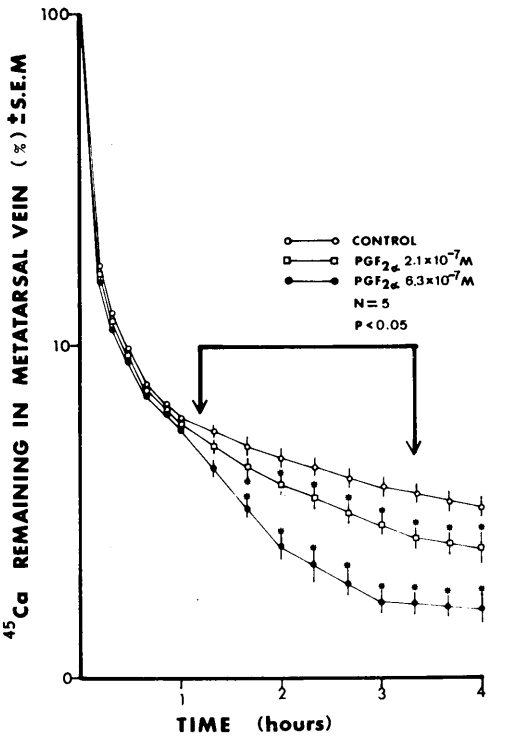

FIG. 4. Effect of $PGF_{2\alpha}$ on calcium 45 efflux from canine metatarsal veins. Ordinate, ^{45}Ca remaining in tissue; abscissa, desaturation time. Means were compared with Student's group *t* test (7). (*) ^{45}Ca efflux in the presence of $PGF_{2\alpha}$ significantly differs ($P < 0.05$) from ^{45}Ca efflux in the absence of prostaglandin $F_{2\alpha}$.

Furthermore, $PGF_{2\alpha}$ facilitates norepinephrine-induced ^{45}calcium uptake into venous smooth muscle. These findings are consistent with the postulate that $PGF_{2\alpha}$ enhances vascular reactivity by a mechanism involving alterations in membrane permeability to calcium ion. Since $PGF_{2\alpha}$ did not increase venous tone these changes probably may not mediate the reported contractile responses to $PGF_{2\alpha}$.

Summary. $PGF_{2\alpha}$ enhanced the contractile responses of superfused helical strips of canine cutaneous venous smooth muscle to norepinephrine and the ionic stimulant, barium chloride, in the absence of any significant effect of $PGF_{2\alpha}$ on venous tone. $PGF_{2\alpha}$ also enhanced norepinephrine-induced ^{45}Ca uptake as well as the rate of ^{45}Ca exchange. It was concluded that $PGF_{2\alpha}$ acts to enhance smooth muscle membrane permeability to calcium ion. In addition, it appears that $PGF_{2\alpha}$ may act within the smooth muscle cell to enhance the rate of transcellular calcium exchange.

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