Effects of Lanthanum on ^{45}Ca Movements and Oxytocin-Induced Milk Ejection in Mammary Tissue (36485)

D. M. LAWSON[1,2] AND G. H. SCHMIDT
(Introduced by William Hansel)
Department of Animal Science, Cornell University, Ithaca, New York 14850

Lanthanum has been used by several investigators to define the functions of various calcium pools within excitable tissues (1–5). This trivalent cation apparently displaces calcium from negatively charged groups on the surface structures of the cell. Autoradiographic evidence (6) indicates that La^{3+} does not move into cells, thus, La^{3+} can directly affect those events normally governed by surface-bound calcium. It has been suggested (5) that La^{3+} also affects the movement and/or binding of calcium at other, less superficial, sites.

Work in this laboratory (7) and others (8, 9) has shown that calcium plays an important role in contraction of myoepithelial cells in the mammary gland. The exact function of calcium remains uncertain, but evidence (7) indicates that multiple sites for calcium are present in myoepithelial cells and the sites may function similarly to those in various types of smooth muscle (10–12). The present study was conducted to investigate the functions of these multiple sites using La^{3+} to isolate effects of different calcium pools.

Three experiments were conducted. The first was to determine if La^{3+} displaces ^{45}Ca from mammary tissue; the second, to determine if oxytocin causes movement of external ^{45}Ca into myoepithelial cells. In the latter experiment lanthanum was used after oxytocin treatment to block subsequent efflux of ^{45}Ca from the intracellular space as described by Van Breeman and McNaughton (1). The third experiment examined the effect of La^{3+} on the milk ejection response of mammary tissue to oxytocin.

Materials and Methods. Experimental animals and tissue preparation. Lactating and involuted mammary tissue from rats was prepared as described previously (7). Involuted mammary tissue was used exclusively in the ^{45}Ca flux experiments to remove the influence of epithelial cells on ^{45}Ca movements. Myoepithelial cells function at the stage of involution used in the present study (13). Lactating tissue was used in the third experiment in which milk ejection was measured.

Experimental Solutions. A Tris-buffered physiological solution (TBPS) with the following chemicals in millimoles per liter of deionized water at pH 7.6 was used; NaCl 150; glucose 5.5; Tris (hydroxymethyl) aminoethane 5; KCl 2.68; $CaCl_2$ 0.92; $MgCl_2$ 0.49; HCl 4.2. Lanthanum when added was at 1 or 2 mmoles per liter. All solutions used in the ^{45}Ca flux experiments were gassed with a 95% O_2-5% CO_2 mixture for 2 min prior to use. Oxytocin (Pitocin, Parke-Davis and Company) was added to the test solutions in Expt. 2 at 10 μU/ml and in Expt. 3 at 100 μU/ml. Egg albumin (0.5%) was added to all oxytocin solutions to prevent binding of the hormone to the glassware. $^{45}Calcium$ was added to the appropriate test solutions in Expts. 1 and 2 at 5 and 3.25 μCi/ml, respectively.

Radioactivity assay and milk ejection response measurements. In the ^{45}Ca flux experiments tissues were blotted, weighed, and extracted using the procedure of Spar-

[1] Present address: Department of Physiology, Wayne State University School of Medicine, 1400 Chrysler Freeway, Detroit, MI 48027.

[2] Supported by NIH Postdoctoral Traineeship (HD-00171-05).

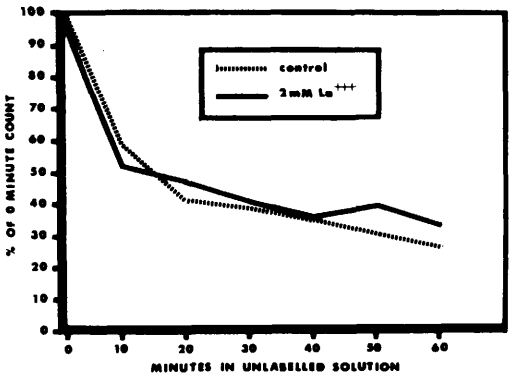

FIG. 1. Effect of lanthanum on ^{45}Ca efflux in rat mammary tissue. Pieces (2 × 2 × 4 mm) of mammary tissue from each of three rats on Day 4 postweaning were placed in TBPS containing 5 μCi ^{45}Ca/ml for 60 min. Then one-half of the pieces were placed in TBPS containing La^{3+} (2 mM), and the remaining pieces were transferred to TBPS. Storage time in these two solutions varied up to 60 min. At each interval four pieces of tissue from each rat were removed from each type of storage media, separately digested, and the digests counted by liquid scintillation spectrometry. The average counts per minute on tissue pieces from each rat at each time interval were taken as a percentage of the 0 minute counts per minute. The mean percentages are shown.

row and Johnstone (14) as described previously (7). The extract (0.5 ml) of each piece of tissue was assayed for ^{45}Ca activity in 10 ml of Bray's solution (15) in a Nuclear-Chicago Mark II Liquid Scintillation Spectrometer. Counts were expressed as counts/min/mg wet tissue.

The response of mammary tissue to oxytocin was assessed using the procedure which Van Dongen and Hays (16) developed as a bioassay for oxytocin. In brief, the procedure involves placing a small piece of lactating rat mammary tissue in a test solution containing oxytocin and measuring the time, in seconds, required for milk ejection to occur. This time is referred to as latency period, and it increases as the response to oxytocin decreases (16). The original procedure was modified slightly as described previously (7).

Statistical analysis. Standard analyses of variance procedures (17) were used throughout this study. Treatment means were compared using Tukey's honestly significant difference procedure (17). The number of observations per treatment are shown in the legends of the figures.

Results and Discussion. The efflux of ^{45}Ca from pieces of involuted mammary tissue previously exposed for 60 min to labeled TBPS (5 μCi ^{45}Ca/ml) was not significantly altered by La^{3+} (2 mM) in the washout media (Fig. 1). Thus, it appears that lanthanum does not displace ^{45}Ca from this tissue. However, both the loading and washout solutions contained 0.92 mM calcium and recent evidence (1–3, 5) indicates that the efflux of ^{45}Ca from various smooth muscle preparations is affected by La^{3+} if nonradioactive calcium is absent from loading and washout solutions. This is probably the case in mammary tissue as well.

A second experiment was conducted to determine if oxytocin causes an influx of extracellular calcium into the myoepithelial cells (Fig. 2). Mammary tissue pieces were exposed to ^{45}Ca TBPS for various periods up to 120 min. At each time interval, representative pieces were exposed for 15 sec to

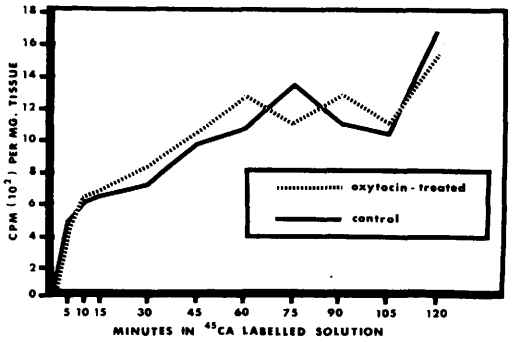

FIG. 2. Effect of oxytoxin on influx of ^{45}Ca in rat mammary tissue. Pieces (2 × 2 × 4 mm) of mammary tissue from each of four rats on Day 4 postweaning were placed in TBPS containing 3.25 μCi ^{45}Ca/ml for varying periods of time up to 120 min. At each interval four pieces of tissue from each rat were removed; two were placed in labeled TBPS for 15 sec; and the other two were placed in labeled TBPS containing 10 μU oxytocin/ml for 15 sec. All four pieces were transferred to unlabeled TBPS containing 1 mM La^{3+} for 30 min. Then all pieces were separately digested and the digest counted by liquid scintillation spectrometry. Each point represents eight observations.

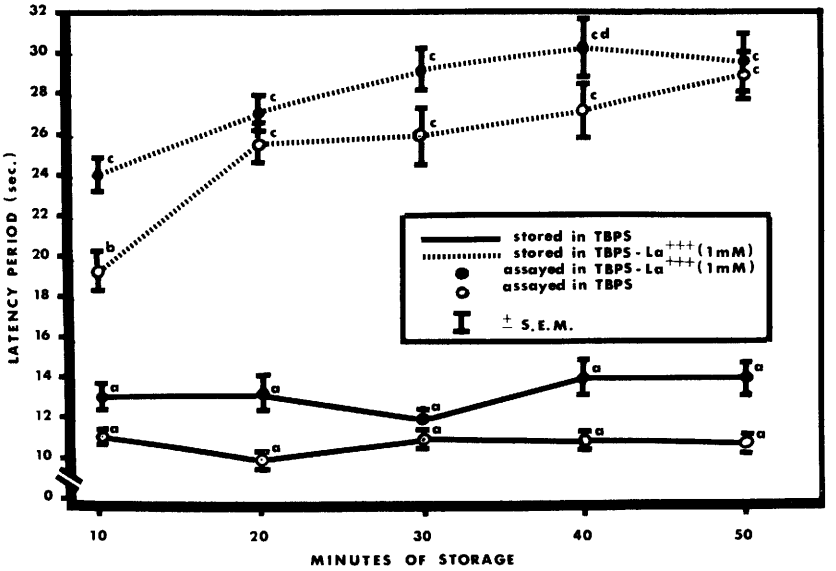

FIG. 3. Effect of lanthanum on latency to milk ejection in rat mammary tissue. Pieces (1 mm^3) of mammary tissue from each of five lactating rats were placed in either TBPS or TBPS containing 1 m*M* La^{3+} for varying periods up to 50 min. At each time interval the latency to milk ejection was measured on each of eight pieces of tissue from each storage treatment; four were assayed in TBPS and four in TBPS containing 1 m*M* La^{3+}. All assay solutions contained 100 μU oxytocin/ml. Each point represents twenty observations. a < b < c < d (h.s.d.; $p = .05$).

either labeled TBPS or labeled TBPS containing 10 μU oxytocin/ml. All pieces were then placed for 30 min in TBPS containing 1 m*M* La^{3+} in an effort to stabilize the ^{45}Ca, which may have moved into the myoepithelial cells. After 10–60 min of loading with ^{45}Ca there was slightly more radioactive calcium in the tissues treated with oxytocin; however, the differences were not significant at $p = 0.05$ (Fig. 2). Therefore, it is still not certain if oxytocin causes myoepithelial cell contraction by producing an influx of extracellular calcium. Earlier evidence (7) indicated that calcium from a cellular pool was at least partially responsible for myoepithelial cell contraction.

The third experiment (Fig. 3) shows that lanthanum suppresses contraction of the myoepithelial cell. In this experiment tissue pieces from lactating rats were stored in TBPS or TBPS containing 1 m*M* La^{3+}, and then the milk ejection response was measured in the same solutions containing 100 μU of oxytocin/ml. Storage in TBPS containing La^{3+} significantly ($p = 0.05$) increased the time required for milk ejection to occur compared to those times observed when tissue was stored in TBPS. When tissue was stored in TBPS and assayed in TBPS-La^{3+}, the latency period increased, although not significantly ($p = 0.05$), compared to the time observed when tissues were assayed in TBPS. When tissue was stored in TBPS-La^{3+} and then assayed in TBPS, the latency decreased significantly ($p = 0.05$) at 10 min of storage. Both of these responses indicate that the effect of La^{3+} on myoepithelial cell contraction occurs rapidly after the ion is presented to the cell (or is withdrawn from the cell). Thus, a cell surface action of lanthanum is indicated as has been proposed previously (1–3). If the action of lanthanum is on the displacement of calcium, the results presented here indicate that superficial calcium is involved. This source of calcium may be involved directly with the contractile apparatus or may control another source of calcium elsewhere in the cell, which in turn regulates the function of the contractile apparatus. This is not yet elucidated.

Summary. Lanthanum did not cause an expected increase in the efflux of ^{45}Ca from mammary tissue. The presence of nonradioactive calcium in the media probably precluded such movement. However, lanthanum did significantly reduce the milk ejection response of mammary tissue to oxytocin. The nature of this reduction indicates that a superficial calcium pool plays a role in myoepithelial cell contraction. Evidence in this report does not strongly favor any direct movement of extracellular calcium into the myoepithelial cell after oxytocin treatment.

1. Van Breeman, C., and McNaughton, E., Biochem. Biophys. Res. Commun. **39**, 567 (1970).

2. Goodman, F. R., and Weiss, G. B., J. Pharmacol. Exp. Therap. **177**, 415 (1971).

3. Goodman, F. R., and Weiss, G. B., Amer. J. Physiol. **220**, 759 (1971).

4. Weiss, G. B., J. Pharmacol. Exp. Therap. **174**, 517 (1970).

5. Weiss, G. B., and Goodman, F. R., J. Pharmacol. Exp. Therap. **169**, 46 (1969).

6. Laszlo, D., Ekstein, D. M., Lewin, R., and Stern, K. G., J. Nat. Cancer Inst. **13**, 559 (1952).

7. Lawson, D. M., and Schmidt, G. H., Amer. J. Physiol. **222**, 444 (1972).

8. Polácek, I., Krejcí, I., and Rudinger, J., J. Endocrinol. **38**, 13 (1967).

9. Moore, R. D., and Zarrow, M. X., Acta Endocrinol. **48**, 186 (1965).

10. Hinke, J. A. M., *in* "Muscle" (W. M. Paul, ed.). Pergamon Press, New York (1965).

11. Hurwitz, L., Von Hagen, S., and Joiner, P. D., J. Gen. Physiol. **50**, 1157 (1967).

12. Van Breeman, C., and Daniel, E. E., J. Gen. Physiol. **49**, 1299 (1966).

13. De Nuccio, D. J., and Grosvenor, C. E., Amer. J. Physiol. **212**, 149 (1967).

14. Sparrow, M. P., and Johnstone, B. M., Biochem. Biophys. Acta **90**, 425 (1964).

15. Bray, G. A., Anal. Biochem. **1**, 279 (1960).

16. Van Dongen, C. G., and Hays, R. L., Endocrinology **78**, 1 (1966).

17. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw Hill Book Company, Inc., New York (1960).

Received Feb. 1, 1972. P.S.E.B.M., 1972, Vol. 140.