Divalent Cation Inhibition of Hormone Release from Isolated Adenohypophysial Secretory Granules*

Mary Y. Lorenson, David L. Robson, and Laurence S. Jacobs

From the Endocrine Metabolism Unit, Department of Medicine, and The Clinical Research Center, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Divalent cations inhibited in vitro release of growth hormone (GH) and prolactin (PRL) from bovine adenohypophysial secretory granules. Zinc, nickel, and cadmium were most potent, exerting 50% inhibition of protein release near 0.1 mM; relative potency was Ni** > Zn** > Cd** > Mn** > Co** > Cu** > Mg** > Ca**. The pH optimum for inhibition, 8.0, was lower than that for stimulation of release by thios. EDTA augmented release and reversed metal inhibition. Both immunocytoassay and polyacrylamide gel electrophoresis results indicated that metals inhibited both PRL and GH release in a dose-related fashion, and that PRL was more sensitive to all cations tested. With zinc present, known stimulators of release (reduced glutathione, ATP, and bicarbonate) restored GH release, but only ATP restored PRL release. Bicarbonate potently stimulated GH release, but only affected PRL when Mg** and ATP were present. We suggest that divalent cations influence GH and PRL release in a reversible fashion and at multiple sites. Some loci may be common to both lactotrope and somatotrope granules; however, the different sensitivities to metals and differential reversal by stimulators of release indicate that metal-protein interactions may also be specific for either granule, or for the hormones themselves.

SECRETORY SITES REQUIRE CALCIUM FOR MAINTENANCE OF NORMAL RESPONSES TO SECRETAGOGUES (1-3). SIMILARLY, CALCIUM IS REQUIRED IN THE RESPONSE OF TARGET TISSUES TO HORMONAL ACTIONS (3, 4). MANY CELLULAR SITES AND BIOCHEMICAL PROCESSES MAY BE INFLUENCED BY CALCIUM; THE CELL MEMBRANE (5-7), MICROTUBULES (8, 9), AND SECRETORY GRANULES (10, 11) ARE INCLUDED AMONG THE CELLULAR LOCI WHERE CALCIUM MAY PLAY IMPORTANT ROLES IN SECRETION. RECENT OBSERVATIONS HAVE SUGGESTED THAT HIGH CALCIUM CONCENTRATIONS MIGHT PLAY A ROLE IN MODULATING THE INHIBITORY EFFECTS OF SOMATOSTATIN ON HORMONE SECRETION, AT LEAST IN THE PANCREAS (12, 13). IN THE PITUITARY, IN CONTRAST, EXCESS CALCIUM TENDS TO SUPPRESS RELEASE OF GH (14), THUS MIMICKING THE EFFECT OF SOMATOSTATIN RATHER THAN COUNTERRACTING IT. EXCESS CALCIUM ALONE HAS BEEN REPORTED EITHER TO SUPPRESS GH RELEASE (15) OR TO BE WITHOUT EFFECT (16). UNDER MOST EXPERIMENTAL CONDITIONS, EXTRACELLULAR CALCIUM REDUCES IN VITRO SECRETION OF PRL AND GH, BUT IS APPARENTLY NOT REQUIRED FOR MAINTENANCE OF THE STIMULATORY EFFECT OF THEOPHYLLINE ON BOTH HORMONES, OR FOR EXPRESSION OF THE INHIBITORY EFFECT OF DOPAMINE ON PROLACTIN SECRETION (17). RECENT STUDIES HAVE SUGGESTED THAT AT LEAST ONE SECRETAGOGUE FOR PRL, THYROTROPIN-RELEASING HORMONE, DOES NOT INDUCE INFUX OF EXTRACELLULAR CALCIUM BUT RATHER MOBILIZES CALCIUM INTO GH, CELL CYTOSOL FROM INTRACELLULAR SITES; SECRETION IS STILL ULTIMATELY DEPENDENT UPON CALCIUM AVAILABILITY (18).

OUR INTEREST IN EXAMINING THE EFFECTS OF CALCIUM AND OTHER DIVALENT CATIONS ON HORMONE RELEASE FROM SECRETORY GRANULES DEVELOPED OUT OF PREVIOUS GRANULE STUDIES DEALING WITH A Mg**-DEPENDENT, ANION-SENSITIVE GRANULE MEMBRANE ATPase (19). IN THE COURSE OF ESTABLISHING THAT OPTIMAL CONDITIONS FOR ENZYME ACTIVITY DIFFERED SUBSTANTIALLY FROM CONDITIONS AUGMENTING HORMONE RELEASE, WE NOTED THAT Mg** WAS A POTENT INHIBITOR OF GH AND PRL RELEASE (20). WE WONDERED WHETHER THE INHIBITION OBSERVED WAS SPECIFIC FOR Mg**, AND IF IT REFLECTED ONE OR MORE REGULATORY PHENOMENA WHICH MIGHT PLAY IMPORTANT ROLES IN THE CONTROL OF SECRETION IN VITRO. IN ORDER TO ADDRESS THESE QUESTIONS, WE HAVE EMPLOYED OUR RECENTLY DEVELOPED SECRETORY GRANULE INCUBATION SYSTEM (21). THIS IN VITRO HORMONE RELEASE SYSTEM MAY SIMULATE THE EVENTS OF THE FINAL STAGE OF SECRETION, THE DISSOLUTION OF STORED GRANULE HORMONE INTO THE EXTRACELLULAR SPACE FOLLOWING FUSION OF THE GRANULE AND PLASMA MEMBRANES. THE PRESENT STUDIES, USING THIS METHOD, INDICATE THAT A WIDE VARIETY OF DIVALENT CATIONS SHARE WITH MAGNESIUM THE ABILITY TO INHIBIT GH AND PRL RELEASE FROM ISOLATED SECRETORY GRANULES, AND THAT THIS INHIBITORY EFFECT IS REVERSIBLE WITH EDTA, AND ALSO COUNTERACTED BY ATP, REDUCED GLUTATHIONE, AND BICARBONATE.

EXPERIMENTAL PROCEDURES AND RESULTS

DISCUSSION

The data reported here indicate that a number of divalent cations are potent inhibitors of the release of GH and PRL from suspensions of secretory granules. No apparent relationship existed between the inhibitory potency of these metals and their size or orbital structures, or their electrophilicity.

*This research was supported in part by Grants AM-21783 and AM-31326 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases and Grant FR-00044 from the Division of Research Resources, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: GH, growth hormone; PRL, prolactin; GSH, reduced glutathione.

2 M. Y. Lorenson and L. S. Jacobs, manuscript submitted for publication.

3 Portions of this paper (including "Experimental Procedures," "Results," Tables 1 and II, and Figs. 1-8) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No, 82M-3289, cite the authors, and include a check or money order for $5.60 per set of photocopies. Full size photocopies are also included in the microform edition of the Journal that is available from Waverly Press.
The independent results of the protein measurements, radioimmunoassay determinations, and polyacrylamide gel patterns corroborated each other. Results in Fig. 1 and Table I showed that metal inhibition was dose-dependent and that PRL release was more sensitive than GH. Data in Table I also showed that EDTA stimulated release of both hormones in the absence of added metals. It should be pointed out that neither metals nor EDTA had any direct effect on the hormonal radioimmunoassays at the concentrations used in the studies. The greater sensitivity of PRL than of GH to metal inhibition was also observed in polyacrylamide gel electrophoresis studies (not shown); for example, at zinc concentrations of 100 μM, only the PRL band became undetectable. Previously, we had also shown that PRL was more sensitive than GH to the stimulatory effects of thiols on hormone release (21). In addition, granule PRL immunoreactivity was tremendously enhanced by thiols, whereas that of GH was only modestly affected (21). This information suggests that the proteins in the membranes of each granule type have different sensitivities, or that the intragranular storage form of PRL is more sensitive to metals and thiols. If the hormones interact directly with the cations, the disulfide near the NH₂ terminus of the molecule, present only in PRL, might be involved, in part, in metal-thiol complexation. (Both PRL and GH have a midmolecule large disulfide loop and a small COOH terminus disulfide.)

Results from the studies in which granules were incubated with a combination of two cations (e.g. zinc and magnesium) were also in agreement with the suggestion that PRL and GH granules have different sensitivities to divalent cations and contain several metal-sensitive sites. First, PRL release was again more potently inhibited than GH release under all conditions. Second, the observed inhibition at submaximal Zn²⁺ and Mg²⁺ concentrations was equal to the additive effects of each alone, whether protein or immunoassay data were employed to evaluate the results (Figs. 2 and 3). Although inferences regarding the similarities or differences of mechanisms of inhibition by the two cations cannot be made from these studies, several sites with different sensitivities are implied since, otherwise, competition with less than additive effects, would have been expected.

Like thiol stimulation of hormone release from granules, the divalent cation inhibition varied with the pH of the medium, being attenuated in the acidic range, and maximal at alkaline values. Thiol-stimulated and basal hormone release were maximal at pH 8.8 in these studies (Fig. 4; also Ref. 21), but inhibition by zinc was maximal at pH 8.0 and decreased somewhat at the higher pH value. While the mechanism of metal inhibition cannot be ascertained from these studies, the metals may interact with critical thiol groups. We have previously suggested that thiol-disulfide interchange reactions may be critical for the release process (21, 27, 28), involving thiols present either in granule membranes or in hormone storage forms. Divalent cations may inhibit these directly or they may inhibit enzymes involved in the interchange process. For example, a pituitary GSH-disulfide oxidoreductase is known which is capable of catalyzing disulfide interchange between GSH and secretory granule protein disulfides (29, 30) and which is inhibitable by metals (30). Stimulation of release by thiols may be under enzymatic control at or below pH 8, whereas nonenzymatic disulfide interchange, being increased in the alkaline range, may account for the increased basal and thiol-stimulated release at pH 8.8. These data, of course, are also consistent with separate mechanisms for thiol stimulation and for metal inhibition of release.

The reversibility of the inhibitory effect of metals by EDTA is clearly shown in Figs. 5 and 6. Although reversibility became less complete as prior incubation time with zinc was prolonged, nonetheless, over 70% of the maximal hormone release was still observed even after 30 min of exposure to zinc. Also, the effect of 1 h of contact with metal, as shown in Fig. 6, is virtually completely reversible in a second incubation with EDTA. Such prompt and substantial reversal of inhibition indicates that a nonspecific denaturation or precipitation of the hormones by metal is quite unlikely. This was also substantiated by electron microscopic studies which indicated that granules incubated with zinc were indistinguishable from those incubated without metals. One of the interesting observations in the two-stage incubation experiment was the failure of EDTA to augment hormone release when it was present only during the second incubation (Fig. 6, top pair of bars). This suggests the possibility that, during the first hour of control incubation, a change may take place in the granule or its contained hormone which renders it indifferent to the stimulatory effects of EDTA. This stands in striking contrast to the marked stimulation of release observed when EDTA was present from the start of the incubation (Fig. 5).

Electrophoretic evidence (Fig. 7) and radioimmunoassay data (Table II and text) indicated that the inhibitory effects of divalent cations could be counteracted by stimulators of release other than EDTA. The mechanisms by which ATP, HCO₃⁻, and thiols stimulate release have not been elucidated in detail, nor do we know conclusively from these data whether the stimulators and metals were acting at the same site(s). However, the finding that GH release and PRL release were similar at comparable GSH:zinc ratios even when absolute concentrations of GSH and zinc varied by 10-fold suggested that these ligands were in fact competing for the same site(s) rather than complexing with each other. Differences in responsiveness of GH and PRL granules were apparent. GSH and HCO₃⁻, added to inhibitory concentrations of zinc, influenced primarily GH release whereas ATP attenuated the zinc inhibition of both hormones. These differences may relate to differences in numbers or characteristics of granule-binding sites for these agents, or differences in the molecular structures and organization of the granule membranes or the hormones. Since bicarbonate had previously been shown to stimulate the release or production of PRL (but not GH) by hemipituitaries or pituitary cells (31–33), additional experiments were carried out with bicarbonate in the presence of magnesium. These results, shown in Fig. 8, indicated that magnesium and bicarbonate could modulate each other’s effect on protein, GH, and PRL release. PRL was more sensitive to Mg²⁺, while GH was more sensitive to HCO₃⁻. In fact, virtually no effect of HCO₃⁻ on PRL was seen unless Mg²⁺ was present, thus implying that different mechanisms were operational for GH and PRL release under these conditions. The divalent cation environment might be critical for expression of the bicarbonate stimulatory effects in PRL granules but such a requirement may not be a major factor in GH release mechanisms.

The absence or diminution of stainable protein bands with zinc exposure (Fig. 7), despite a comparable Lowry protein load in each lane of the gel, coupled with increases in stainable protein at the gel origin, constitutes strong circumstantial evidence for an induced increase in molecular size under the influence of metals, or an inhibition of conversion to smaller forms. Such an effect might be expected to cause a reduction in immunoassayable hormone even if these large forms are released, since very high molecular weight disulfide oligomers of both GH and PRL have been noted to have poor immu-

* M. Y. Lorenson and L. S. Jacobs, unpublished observations.
noactivity on a protein basis (27, 28, 34). This information from the nondenaturing gel runs shown in Fig. 7 was buttressed by identical results when supernatants from metal-exposed granules were electrophoresed in sodium dodecyl sulfate (not shown). Other investigators have reported that nickel inhibits the secretion of PRL but not that of other adenohypophysial hormones, from pieces of tissue incubated in vitro (35, 36). The present data, which show that nickel potently inhibits GH as well as PRL release from isolated granules, may not necessarily contradict the prior data, since the systems used are so different. Obviously, many other sites are potentially available in whole tissues including plasma membrane sites which may complex the cations, thus preventing their internalization.

In summary, these experiments demonstrate that divalent cations strikingly inhibit GH and PRL release from incubated secretory granules in a reversible and dose-related fashion. Given the differences observed with incubation time and sequence of addition of effective compounds, the interactions with glutathione, ATP, and bicarbonate, the additivity of combinations of cations, the differential magnitude of the effects on PRL and GH, and the electrophoretic evidence for changes in hormonal molecular size with cations, it seems that a multiplicity of sites may be involved in the expression of these effects, as is the case with thiol stimulation of hormone release (21).

REFERENCES

1. Douglas, W. W. (1979) Biochem. Soc. Symp. 39, 1–28
2. Parsons, J. A. (1970) J Physiol. (Lond.) 210, 973–987
3. Steiner, A. L., Peake, G. T., Utiger, R. D., Karl, I. E., and Kipnis, D. M. (1970) Endocrinology 86, 1354–1360
4. Rasmussen, H., and Goodman, D. B. P. (1977) Physiol. Rev. 57, 421–509
5. Hoffstein, S. (1979) J. Immunol. 123, 1395–1402
6. Naccache, P. H., Showell, H. J., Becker, E. L., and Sha’afi, R. I. (1979) J. Cell Biol. 83, 179–186
7. Gershengorn, M. C., Hoffstein, S. T., Rebecchi, M. J., Geras, E., and Rubin, I. (1981) J. Clin. Invest. 67, 1799–1776
8. Allison, A. C., and Davies, P. (1974) Adv. Cytoskepmacl. 2, 237–248
9. Sherline, P., Lee, Y-C., and Jacobs, L. S. (1977) J. Cell Biol. 72, 380–389
10. Creutz, C. E., Scott, J. H., Pazoles, C. J., and Pollard, H. B. (1982) J. Cell. Biochem. 18, 87–97
11. Scherman, D., Roisin, M.-P., Henry, J. P., and Jeminet, G. (1981) Biochem. Pharmacol. 30, 3277–3283
12. Curry, D. L., and Bennett, L. L. (1974) Biochem. Biophys. Res. Commun. 60, 1015–1019
13. Bhathena, S. J., Ferrino, P. V., Voyles, N. R., Smith, S. S., Wilkins, S. D., Croy, D. H., Schally, A. V., and Recant, L. (1976) Diabetes 25, 1031–1040
14. Stachura, M. E. (1983) Am. J. Physiol., in press
15. Gauvitik, K., and Tashjian, A. H., Jr. (1973) Endocrinology 92, 573–583
16. Hayasaka-Kimura, N., and Takahashi, K. (1979) Proc. Soc. Exp. Biol. 161, 312–318
17. MacLeod, R. M., Nagy, I., Logan, I. S., Kimura, H., Valdenegro, C. A., and Thorner, M. O. (1980) in Central and Peripheral Regulation of Prolactin Function (MacLeod, R. M., and Scapagnini, U., eds) pp. 27–42, Raven Press, New York
18. Geras, E., Rebecchi, M. J., and Gershengorn, M. C. (1982) Endocrinology 110, 901–906
19. Lorenson, M. Y., Lee, Y-C., and Jacobs, L. S. (1981) J. Biol. Chem. 256, 12802–12810
20. Lorenson, M. Y., and Jacobs, L. S. (1982) Endocrinology 110, (suppl.) 287
21. Lorenson, M. Y., and Jacobs, L. S. (1982) Endocrinology 110, 1164–1172
22. Machlin, L. J., Jacobs, L. S., Cirulis, N., Kimes, R., and Miller, R. (1974) Endocrinology 95, 1350–1358
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
24. Ornstein, L. (1984) Ann. N. Y. Acad. Sci. 121, 321–349
25. Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427
26. Merril, C. R., Goldman, D., and Van Keuren, M. L. (1982) Electrophoresis 3, 17–23
27. Lorenson, M. Y., Lee, Y-C., Miska, S. P., and Jacobs, L. S. (1983) in Frontiers and Perspectives of Prolactin Secretion: A Multi-disciplinary Approach (Mena, F., and Valverde, C., eds) Academic Press, New York, in press
28. Jacobs, L. S., and Lee, Y-C. (1975) Endocrinology 96, (suppl.) 83
29. Lorenson, M. Y., Lee, Y-C., and Jacobs, L. S. (1981) Life Sci. 28, 2309–2315
30. Lorenson, M. Y., and Jacobs, L. S. (1981) Biochim. Biophys. Acta 661, 63–73
31. MacLeod, R. M., and Fontana, E. H. (1970) Endocrinology 86, 863–869
32. Lamberts, S. W., and MacLeod, R. M. (1979) Proc. Soc. Exp. Biol. Med. 161, 495–497
33. Wilfinger, W. W., Davis, J. A., Augustine, E. C., and Hymer, W. C. (1970) Endocrinology 105, 530–536
34. Sigel, M. B., Vanderlaan, W. P., Vanderlaan, E. F., and Lewis, U. J. (1980) Endocrinology 106, 92–97
35. LaBella, F., Dular, R., Vivian, S., and Queen, G. (1973) Biochem. Biophys. Res. Commun. 52, 786–791
36. LaBella, F., Dular, R., Lemos, F., Vivian, S., and Queen, G. (1973) Nature (Lond.) 245, 390–392
Divalent Cation Inhibition of Hormone Release from Granules

IMETILI, mM

PRL

81.5

51.9

43.5

GH

111.9

89

4

60.4

CO+2

EDTA

-2

50

pY

ZnCl2

n

6.5 7.2 8.0 8.8

DH

by guest on March 17, 2020
http://www.jbc.org/ Downloaded from

Figure 1. Inhibition of protein release by metals.

Figure 2. Comparison of actual and expected release values with combinations of cations. Data from Figure 2, as well as protein release data, are included. The interrupted line is the theoretical line of identity. The solid line is the least-squares line of all data points. The correlation coefficients for the least-squares line and for the correlation of actual vs. expected release are 0.95 and 0.96, respectively.

Figure 3. Comparison of actual vs. expected release values with combinations of cations. Data from Figure 2, as well as protein release data, are included. The interrupted line is the theoretical line of identity. The solid line is the least-squares line of all data points. The correlation coefficients for the least-squares line and for the correlation of actual vs. expected release are 0.95 and 0.96, respectively.

Effect of pH or Metal Inhibition and Stimulation of Hormone Release: Previous experiments had been carried out at pH 6.5 by adjusting the pH of all solutions to a constant value of 6.5 or 5.5. We therefore compared the influence of pH on release in the presence of 50 μM Zn²⁺ (upper trace) with that at pH 6.5 (lower trace). As seen in Figure 4, in the absence of Zn²⁺, release was negligible at both pH values. At all pH values, release was increased by the addition of Zn²⁺, indicating that Zn²⁺ inhibited the release of both basal and EDA-stimulated release. Furthermore, the inhibition was greater at lower pH values, as seen in Figure 4. We therefore conclude that the release of hormone from granules is pH-dependent, and that the effect of Zn²⁺ is pH-dependent as well.

Effect of pH or Metal Inhibition and Stimulation of Hormone Release: Previous experiments had been carried out at pH 6.5 by adjusting the pH of all solutions to a constant value of 6.5 or 5.5. We therefore compared the influence of pH on release in the presence of 50 μM Zn²⁺ (upper trace) with that at pH 6.5 (lower trace). As seen in Figure 4, in the absence of Zn²⁺, release was negligible at both pH values. At all pH values, release was increased by the addition of Zn²⁺, indicating that Zn²⁺ inhibited the release of both basal and EDA-stimulated release. Furthermore, the inhibition was greater at lower pH values, as seen in Figure 4. We therefore conclude that the release of hormone from granules is pH-dependent, and that the effect of Zn²⁺ is pH-dependent as well.

2.0 4.0 6.0 8.0 10.0

PH

1.0 2.0 3.0 4.0 5.0

INHIBITION

EFFECT

Zn²⁺

EDTA

GSH

0.0 0.1 0.2 0.3 0.4

INHIBITION

EFFECT

Zn²⁺

EDTA

GSH

0.0 0.1 0.2 0.3 0.4

Table 1

Figure 4. Influence of pH on basal, stimulated, and metal-inhibited protein release. Secretory granules were isolated under basal conditions for the experiments, or with 50 μM Zn²⁺ or 50 μM EDTA added to the solution. Table 1. Hormone release in the Presence of Divalent Cations. When cations were used alone, basal release was measured in the absence of all other cations. When cations were used in conjunction with EDA, basal release was measured in the absence of all other cations. When cations were used in conjunction with EDA, basal release was measured in the absence of all other cations. When cations were used in conjunction with EDA, basal release was measured in the absence of all other cations. When cations were used in conjunction with EDA, basal release was measured in the absence of all other cations. When cations were used in conjunction with EDA, basal release was measured in the absence of all other cations.
Divalent Cation Inhibition of Hormone Release from Granules

Figure 5A. Reversibility of 
Zn²⁺ inhibition of protein release by EDTA. Granules were incubated for 1 hr alone or with 100 µM Zn²⁺ or Ni²⁺ (Stage I) 
then pellet grumes were resuspended and reincubated with or without 100µM EDTA. No detectable protein was 
released in the second incubation with no additional initial incubation with 
Ni²⁺.

Figure 6. Reversal of metal inhibition of protein release by EDTA. The percentage of protein released was monitored by polyacrylamide gel electrophoresis under non-denaturing conditions and by radioimmunoassay. These results are shown in Table I and Table II, respectively. The figure clearly shows that both GH and PRL bands were lost in the presence of 200 µM Zn²⁺ with all stimulable remaining at the origin. Electrophoresis in SDS-containing gel system (not shown) revealed stimulable protein appearing only at the origin unless detergents were present. The immunoreactive data show a similar dramatic inhibition of release by added Zn²⁺, being greater than 90% for both hormones. When granules were incubated without Zn²⁺, 0.01% Triton X-100, and 10 µM EDTA present, no Zn²⁺ or Ni²⁺ were released. In contrast, 60 µM bicarbonate restored release of both hormones had been blocked. Incorporation of 50 µM Zn²⁺ or Zn²⁺ also restored release of 10 µM Zn²⁺. As with release of biocarbonate, stimulation by Zn²⁺ was less effective in countering the zinc inhibition than in GH release amount 10 µM Zn²⁺ controlled. In contrast, the treated granules with 10 µM Zn²⁺ partially reversed the zinc inhibition, but release was still less than half of the control rate.

Table I: Protein and Hormone Release by Zn²⁺ and Various Stimulators

| Conditions | 100 | 5 µM ATP | 50 µM HCO₃⁻ | 5 µM CSH |
|------------|-----|----------|-------------|---------|
| Zn²⁺       | 100 | 111      | 121         | 104     |
| Ni²⁺       | 100 | 101      | 120         | 103     |

Experiments were also carried out in which the concentrations of sodium bicarbonate 
were in some cases 10-fold higher than those in Table I and Table II, with the ratio of Zn²⁺ to 
molar of ATP remaining the same. When granules were incubated with 50 µM CSH, 50 µM ATP, or 50 µM HCO₃⁻, protein 
release was markedly inhibited (59%, 67%, and 51% of control, respectively). Further 
addition of ATP resulted in only a partial increase of release (more than 65%). As a result, 
addition of M₃ ATP was not significantly different from that observed in Table II. 
Overall, this study provided a basis for understanding the mechanism of 
stimulation of hormone release by various conditions and conditions in which 
the release of different hormones was observed.

Figure 7. Polyacrylamide gel electrophoresis of zymogen granules. Stimulated 
granules were incubated at pH 5.9, using 
5.50 mM acid phosphatase. The zymogen 
was precipitated with 5% trichloroacetic 
acid. All samples were similarly stained. 

Figure 8. Influence of bicarbonate on release (stimulated by M₃ ATP). Granules 
were incubated as described in Table I. Figure 4, and Figure 5, and 
stimulated with ATP, except that 2.5 µM ATP was also included in the incubations. 
Bicarbonate was also incubated under all conditions except in the case of PRL, with no added M₃ ATP, 
while total M₃ ATP concentration.
Divalent cation inhibition of hormone release from isolated adenohypophysial secretory granules.
M Y Lorenson, D L Robson and L S Jacobs

J. Biol. Chem. 1983, 258:8618-8622.

Access the most updated version of this article at http://www.jbc.org/content/258/14/8618

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/14/8618.full.html#ref-list-1