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Ability of Several Cations to Promote Secretion of Thyrocalcitonin in the Pig[1,2] (38559)

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(Introduced by Roy V. Talmage)

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It is well known that hypercalcemia promotes the secretion of thyrocalcitonin (TCT) from the mammalian thyroid gland (1). In addition to calcium two other divalent cations, magnesium and strontium, have been shown either to promote release of TCT from pig thyroid slices *in vitro* (2, 3) or to stimulate secretion of TCT from the pig thyroid gland *in vivo* (4–6). However, since large nonphysiological doses of magnesium or strontium were required to evoke TCT release and since neither of these two cations appeared to be as effective as calcium in promoting TCT release, only calcium appears to be of any importance physiologically in mediating secretion of TCT (1–6).

This report presents findings which show that several cations can stimulate secretion of porcine TCT. The results confirm the earlier reports that magnesium and strontium are able to stimulate TCT release. In addition, the results indicate that large, supraphysiological amounts of two other cations, potassium and barium, also can cause release of TCT and that TCT secretion in the pig does not appear to be stimulated by increases in either sodium or phosphate in the blood.

Materials and Methods. Animals. Young pigs of either sex weighing approximately 20 kg were obtained locally. The animals were fasted for the 24–48 hr period just before the experiments and allowed only tap water.

The experimental procedures involving surgical isolation of the thyroid gland, continuous collection of thyroid venous effluent blood from the heparinized animal, and handling of collected plasma samples have been described previously in detail (7, 8). Thyroid venous blood was collected continuously for periods of 3.5–4 hr in vessels kept on ice. Each sample used for assay represented the total amount of blood collected over a 10–15 min interval. Plasma was obtained by centrifugation at 4° and stored at −20° until assayed. Femoral arterial blood samples also were obtained periodically throughout each experiment; the plasma, obtained by centrifugation, was analyzed for blood electrolytes. In order to help compensate for extracellular fluid lost during collection of thyroid venous blood, 10% dextran (Gentran-40) in 5% dextrose (w/v) (Travenol Labs, Inc., Morton Grove, IL) was infused (0.6–1 ml/min, femoral vein) during a major portion of each experiment.

Test infusions. For infusions, the following isotonic solutions were employed: 0.15 *M* NaCl, 0.15 *M* $MgSO_4$, 0.15 *M* KCl, 0.1 *M* $CaCl_2$, 0.1 *M* $SrCl_2$, and 0.1 *M* $BaCl_2$. To test effects of hyperphosphatemia, 0.3 *M* NaH_2PO_4 was used. The infusions in each experiment all were given via indwelling catheters either systemically (femoral vein) or directly into the thyroid arterial circulation. For systemic infusions, solutions were administered at a rate of 4.5 ml/min using a variable speed, peristaltic pump (Process and Instruments, N.Y.); for thyroid arterial infusions, solutions were administered at a rate of 0.5 ml/min using a Braun Syringe pump (Bronwill Scientific, Rochester, NY). In each experiment test solutions were infused at the same rate for approximately the same length of time (10–12 min).

Analyses. All plasma samples were ob-

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tained by centrifugation within 1 hr after blood collection. Total calcium (9) and phosphorus (10, as modified by Gitelman (unpublished), were determined by automated (AutoAnalyzer) procedures. Sodium and potassium were determined by flame photometry (Patwin Model FC, National Instrument Labs, Washington, D.C.). Plasma samples were not analyzed directly for strontium or barium, which normally are not present in plasma in appreciable quantities; however, since both cations react in the method used to measure calcium (9 and Gitelman, personal communication), the fact that plasma levels of strontium and barium indeed were elevated during the test infusions could be ascertained indirectly (Fig. 1 and Table 2B).

Hematocrit values (percent packed cell volumes) were determined for thyroid venous blood samples. Whole blood was drawn into heparinized microhematocrit tubes, centrifuged for 2 min in a Model MB microhematocrit centrifuge (International Equipment Co., Needham Heights, MA), and read using a Model CR microhematocrit tube reader (International Equipment Co.).

Concentrations of TCT in all thyroid venous plasma samples were determined by radioimmunoassay. Details of the procedure for the radioimmunoassay of porcine TCT have been described previously (8, 11). Each plasma sample was assayed at multiple dilutions in diluent buffer containing aprotinin (Trasylol, F.B.A. Pharmaceuticals, New York, NY), 300–500 kIU/ml. Assays were conducted under equilibrium conditions at 4° for 48–72 hr using a guinea pig antiserum to porcine TCT (final dilution 1:100,000–1:200,000). Pure porcine TCT was used both as unlabeled standard and for labeling with ^{125}I. Antibody bound and free labeled hormone were separated using dioxane (12). Intra- and interassay variations, as reported previously (13), were no more than 15 and 20%, respectively.

Results. Figure 1 and Table I present results of experiments in which the test solutions were infused directly into the thyroid arterial circulation of two pigs. The results show that large increases in the concentration of TCT in thyroid venous blood were produced during infusions of potassium, strontium, and barium as well as calcium (Fig. 1A, Table I), Lesser elevations in TCT were produced by magnesium administration. The results in Fig. 1B, 1C, and Table I further show that during the infusions, with the exception of plasma sodium, the plasma concentration of each ion tested rose substantially in the blood traversing the thyroid gland (but not in the peripheral, femoral arterial blood). Plasma levels of strontium and barium, although not measured directly (see Methods), rose markedly during their infusion as revealed by the results of the calcium analysis (Fig. 1C). Although plasma sodium concentrations did not increase during infusion of isotonic sodium chloride (Fig. 1B, Table I), the thyroid gland was subjected to hypernatremia during infusion of a large dose of sodium phosphate into the thyroid artery (Table 1, Treatment e). During this test infusion thyroid venous TCT did not increase above the control level despite the fact that both hypernatremia and hyperphosphatemia had been produced. In fact, TCT levels appeared to fall slightly during this infusion probably because of a slight lowering of the thyroid venous plasma calcium level caused by dilution of plasma with the infusate. In the experiments shown in Fig. 1 and Table I it seems likely that increases in TCT were produced directly by the cations being infused since (a) the cations were administered directly into the thyroid artery, and (b) during each infusion the thyroid venous plasma level of the ion being tested rose while levels of the other ions measured either remained unaltered or decreased due to dilution of the thyroid arterial blood by the infusate.

Table II shows results of two related experiments in which the test solutions were infused systemically rather than into the thyroid artery. In these experiments also, infusions of magnesium and strontium as well as calcium elevated thyroid venous TCT concentrations while infusions of sodium and phosphate were ineffective in this regard. Systemic infusions of potassium (Table II), unlike infusion of potassium into the thyroid artery (Fig. 1 and Table I) did not greatly increase plasma potassium and also were not

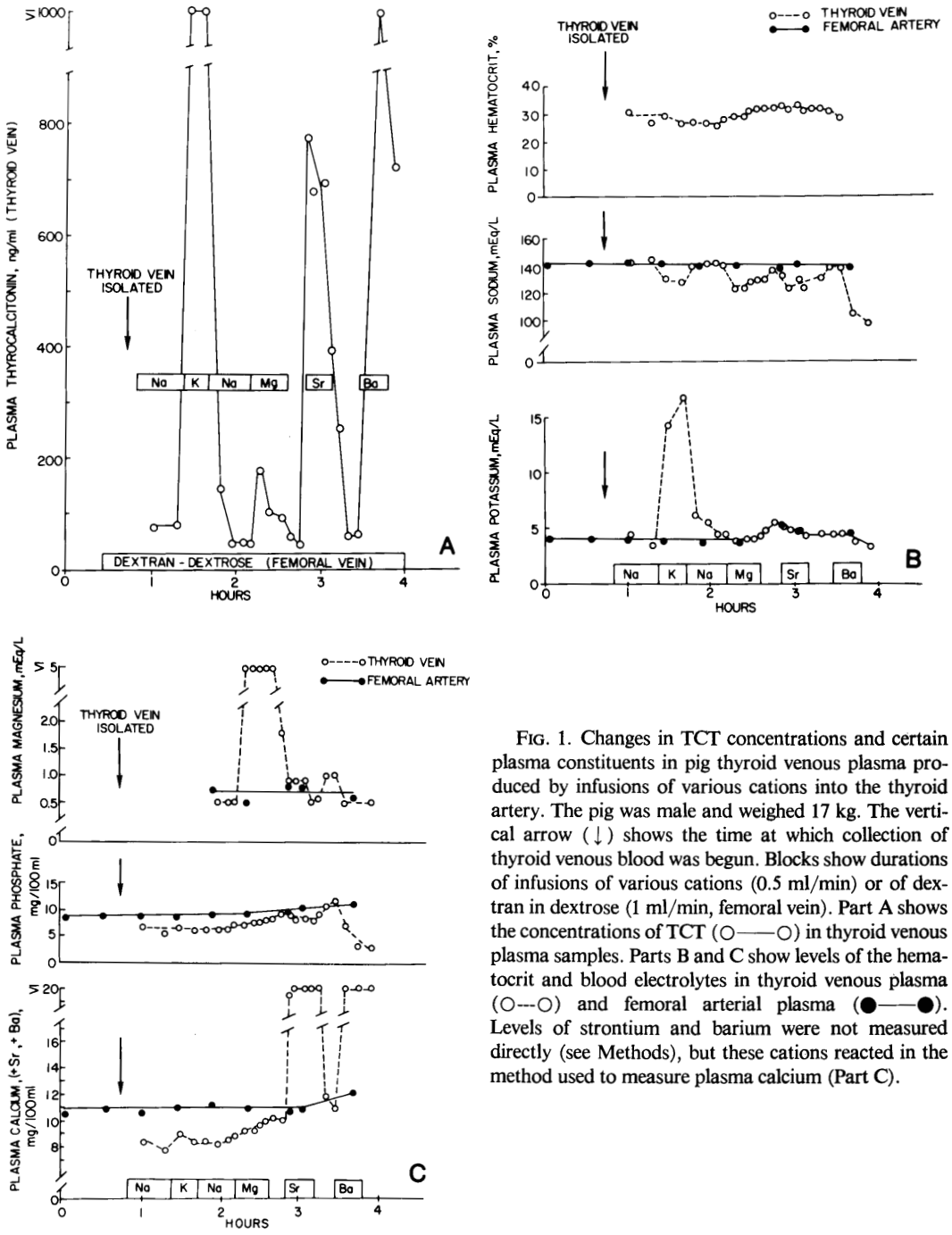

FIG. 1. Changes in TCT concentrations and certain plasma constituents in pig thyroid venous plasma produced by infusions of various cations into the thyroid artery. The pig was male and weighed 17 kg. The vertical arrow (↓) shows the time at which collection of thyroid venous blood was begun. Blocks show durations of infusions of various cations (0.5 ml/min) or of dextran in dextrose (1 ml/min, femoral vein). Part A shows the concentrations of TCT (○——○) in thyroid venous plasma samples. Parts B and C show levels of the hematocrit and blood electrolytes in thyroid venous plasma (○---○) and femoral arterial plasma (●——●). Levels of strontium and barium were not measured directly (see Methods), but these cations reacted in the method used to measure plasma calcium (Part C).

very effective in elevating thyroid venous TCT levels. Table II pig B shows that infusion of the active cations for only 10 min did not stimulate TCT secretion maximally, because prolonging the calcium infusion (from 10 to 22 min), and thereby further elevating the blood calcium level (from 12.2 to 17.0 mg/100 ml), produced an additional fourfold increase in TCT release.

Discussion. The results of these studies

TABLE I. CHANGES IN TCT SECRETION AND CERTAIN PLASMA CONSTITUENTS PRODUCED BY INFUSIONS OF VARIOUS CATIONS OR SODIUM PHOSPHATE INTO THE THYROID ARTERY.*

	Thyroid venous plasma							Femoral arterial plasma				
Treatment	TCT (ng/nl)	Hct[a] (%)	Na (mEq/L)	Mg (mEq/L)	K (mEq/L)	P (mg/100 ml)	Ca (mg/100 ml)	Na (mEq/L)	Mg (mEq/L)	K (mEq/L)	P (mg/100 ml)	Ca (mg/100 ml)
(a) None (control baseline)	40	34.1	135.9	1.58	4.4	8.3	10.6	136.4	1.48	4.1	8.0	10.2
	±2.8[b]	±0.60	±0.30	±0.02	±0.34	±0.06	±0.03	±1.8	±0.09	±0.02	±0.07	±0.10
(b) Na	33	27.4	*135.8*	1.25	3.7	6.4	8.5	*136.8*	1.50	4.4	8.1	10.4
(c) Mg	48	27.4	122.0	*20.0*	3.9	7.6	9.8	135.9	*1.50*	4.4	8.6	10.6
(d) K	801	25.9	132.5	1.90	*14.9*	6.8	8.3	135.0	1.50	*4.5*	8.9	10.7
(e) P (Na)	28	26.5	(165.0)	1.35	4.6	*>20*	9.3	135.0	1.70	4.8	*10.0*	10.7
(f) Ca	417	27.4	136.0	1.40	4.8	16.4	*17.3*	135.0	1.65	4.6	10.6	*10.6*

* The solutions, administered by infusion (0.5 ml/min × 10 min) into the thyroid artery, were 0.15 *M* NaCl, 0.15 *M* $MgSO_4$, 0.15 *M* KCl, 0.3 *M* NaH_2PO_4, and 0.1 *M* $CaCl_2$.

[a] Hct = Hematocrit.

[b] Value presented represents the mean (± SE) of three separate control baseline plasma samples collected at 10–15 min intervals at the beginning of the experiment before infusions were begun; other values represent estimates from single samples collected at 10–15 min intervals during test infusions. Tests were performed sequentially in the order listed. Italicized values show concentrations of each ion in thyroid venous and femoral arterial plasma during infusion of that ion. Value in parenthesis indicates elevated blood level of Na during infusion of sodium phosphate. The pig was female and weighed 18.3 kg.

TABLE II. CHANGES IN TCT SECRETION AND CERTAIN PLASMA CONSTITUENTS PRODUCED BY SYSTEMIC INFUSIONS OF VARIOUS CATIONS OR SODIUM PHOSPHATE.*

	Thyroid venous plasma						
Treatment	TCT (ng/ml)	Hct[a] (%)	Na (mEq/L)	Mg (mEq/L)	K (mEq/L)	P (mg/100 ml)	Ca (mg/100 ml)
Pig A:							
(a) None (Control baseline)	36	36.2	138.3	1.50	4.2	8.7	10.5
	±1.8[b]	±0.37	±0.33	±0.03	±0.03	±0.06	±0.03
(b)	40	37.3	*138.0*	1.55	4.2	8.9	10.5
(c) Mg	131	38.2	137.0	*4.80*	4.2	8.8	10.6
(d) K	49	35.8	137.5	2.65	*5.2*	9.6	10.5
(e) P (Na)	26	35.3	140.0	2.60	5.0	*>20*	10.0
(f) Ca	141	33.8	138.5	2.60	5.0	>20	*12.4*
Pig B:							
(a) None (Control baseline)	38	31.5	130.8	1.23	4.8	9.1	10.5
	±1.5	±0.17	±0.17	±0.03	±0.09	±0.03	±0.03
(b) Na	33	30.8	*131.0*	1.20	4.8	9.1	10.4
(c) Mg	70	31.2	130.0	*4.30*	4.4	9.1	10.4
(d) K	36	29.8	129.5	3.00	*5.8*	9.6	10.3
(e) Sr	126	30.8	128.5	2.40	5.4	10.3	(12.1)
(f) Ca_1	151	29.2	124.0	1.80	5.4	12.5	*12.2*
(g) Ca_2	604	29.2	124.5	1.70	5.1	11.0	*17.0*

* The solutions, administered by infusion (4.5 ml/min × 10–12 min) into a femoral vein, were 0.15 *M* NaCl, 0.15 *M* $MgSO_4$, 0.15 *M* KCl, 0.1 *M* $SrCl_2$, 0.3 *M* NaH_2PO_4 and 0.1 *M* $CaCl_2$. In (g), Ca was infused at 4.5 ml/min × 22 min.

[a] Hct = Hematocrit.

[b] Value presented represents the mean (± SE) of three to four separate control baseline plasma samples collected at 10–15 min intervals at the beginning of the experiment before infusions were begun; other values represent estimates from single samples collected at 10–15 min intervals during test infusions. Tests were performed sequentially in the order listed. Italicized values show plasma concentrations of each ion during infusion of that ion. Values in parenthesis indicate elevated blood levels of Sr as detected by the Ca analysis (see Methods). The pigs were female (A = 22.0 kg, B = 16.9 kg).

agree with earlier reports (2–6) in showing that, in addition to calcium, the divalent cations magnesium and strontium can promote release of TCT from the pig thyroid gland (Fig. 1 and Table II). The fact that an extremely high, unphysiological blood level of magnesium (20 mEq/L, Table I) did not necessarily produce a significant increase in TCT is in accord with the findings of Care *et al.* (4) who reported stimulation of TCT secretion with increases in blood magnesium up to 7 mEq/L, but a reversal of the stimulatory effect with blood magnesium levels greater than 7 mEq/L.

The present results further show that the cations potassium and barium also are capable of promoting TCT release. Whether all of the effective cations tested act on the thyroid gland by the same or different mechanisms is not known. However, the ability of barium to increase TCT (Fig. 1) may be related to the fact that, like calcium, magnesium and strontium, it is a divalent cationic alkaline-earth metal. On the other hand, potassium, which was effective only when delivered in large amounts (lethal blood levels) directly into the thyroid arterial circulation (Fig. 1 and Table I) may have produced TCT release by its well-known ability to depolarize the cell membrane. Large amounts of potassium added *in vitro* to cell cultures of endocrine tissues, e.g., pituitary cell cultures (14), are known to trigger secretion of peptide hormones. In the present study high levels of potassium produced no obvious permanent C-cell damage, since the secretion of TCT returned to baseline following infusion of potassium, and the thyroid gland subsequently responded to infusions of other stimulatory cations (Fig. 1 and Table I).

Of interest is the finding that neither hypernatremia (Table I) nor hyperphosphatemia (Tables I and II) noticeably affected TCT release. Thus, like secretion of parathyroid hormone, secretion of TCT does not appear to be influenced directly by blood phosphate itself, although hyperphosphatemia sufficiently severe to lower blood calcium may indirectly affect release. The findings with phosphate are of special interest in view of the proposal of Talmage and coworkers (15, 16) that the well known hypocalcemic effect of TCT may be mediated by a prior action of the hormone to alter the blood phosphate level.

Although the present findings do show that several cations in addition to calcium can stimulate TCT release, more critical studies would be required to establish the order of effectiveness of these cations. Others have reported calcium to be more effective than magnesium or strontium (4, 6). It is possible that in some of the experiments reported here (Fig. 1 and Table I) the degree of stimulation was influenced by the fact that blood levels of certain ions were reduced during infusion of another ion. Nevertheless, these same experiments, involving infusion of the test ions directly into the thyroid artery, served to demonstrate a direct effect of the test ion on the thyroid gland and not via some other known TCT secretagogue such as, for example, gastrin (13).

These studies support the suggestions of others (2–6) that cations other than calcium, although able to stimulate TCT release, probably do not normally influence TCT secretion to any great extent. High, nonphysiological doses of these ions appeared to be required to promote TCT release, and in some cases (e.g., potassium) these high levels were in the lethal range. Nevertheless the findings demonstrate that several alkaline-earth cations and the alkali metal, potassium, can produce release of TCT from the pig thyroid gland. These agents may be of use as pharmacological tools in exploring the mechanisms involved in the secretion of TCT.

Summary. Various ions were tested to see whether or not at pharmacological plasma levels they affected TCT secretion from the pig thyroid gland *in vivo*. Test solutions were infused either systemically (femoral vein) or directly into the thyroid artery for brief (10–12 min) periods. Infusions of large doses of magnesium, potassium, strontium and barium, as well as calcium, produced increases in TCT in thyroid venous plasma ranging from two- to tenfold. Blood analyses revealed that levels of these stimulatory cations produced in plasma during the infusions indeed were high. In contrast, infusions of sodium and phosphate suggested that

neither hypernatremia nor hyperphosphatemia directly altered TCT secretion.

The findings are in accord with previous suggestions that although cations other than calcium are capable of increasing TCT secretion, probably only calcium plays an important role in the physiological regulation of TCT secretion. Nevertheless, other effective agents, such as those reported here, may constitute useful pharmacological tools for studying the mechanisms involved in secretion of TCT.

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1. Hirsch, P. F., and Munson, P. L., Physiol. Rev. **49,** 548 (1969).
2. Radde, I. C., Parkinson, D. K., Witterman, E. R., and Hoffken, V., *in* "Calcitonin 1969" (S. Taylor, ed.), p. 376. Wm. Heinemann Ltd., London (1970).
3. Bell, N. H., J. Clin. Invest. **49,** 1368 (1970).
4. Care, A. D., Bell, N. H., and Bates, R. F. L., J. Endocrinol. **51,** 381 (1971).
5. Littledike, E. T., and Arnaud, C. D., Proc. Soc. Exp. Biol. Med. **136,** 1000 (1971).
6. Pento, J. T., Glick, S. M., Kagan, A., and Gorfien, P. C., Endocrinology **94,** 1176 (1974).
7. Care, A. D., Cooper, C. W., Duncan, T., and Orimo, H., Endocrinology **83,** 161 (1968).
8. Cooper, C. W., Deftos, L. J., and Potts, J. T., Jr., Endocrinology **88,** 747 (1971).
9. Gitelman, H. J., Anal. Biochem. **18,** 521 (1967).
10. Chen, P. S., Jr., Toribara, T. Y., and Warner, H., Anal. Chem. **28,** 1756 (1956).
11. Deftos, L. J., Lee, M. R., and Potts, J. T., Jr., Proc. Nat. Acad. Sci. USA **60,** 293 (1968).
12. Deftos, L. J., Metabolism **20,** 1122 (1971).
13. Cooper, C. W., Schwesinger, W. H., Ontjes, D. A., Mahgoub, A. M., and Munson, P. L., Endocrinology **91,** 1079 (1972).
14. Vale, W., Grant, G., Amoss, M., Blackwell, R., and Guilleman, R., Endocrinology **91,** 562 (1972).
15. Kennedy, J. W., and Talmage, R. V., Endocrinology **88,** 1023 (1971).
16. Talmage, R. V., Whitehurst, L. A., and Anderson, J. J. B., Endocrinology **92,** 792 (1973).

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