tion) with the characteristics of a squid axon 500 μ in diameter. This would appear to represent a biological example of an optimal match (equality) of source to load for maximum power transfer.

The observation that the transmitter equilibrium potential in this synapse is at, or close to, E_{Na} suggests that the synaptic conductance change is due predominantly to a change in sodium conductance. This contrasts markedly with the effect of acetylcholine at the frog neuromuscular junction where an increase in both sodium and potassium conductance occurs. Here, because the increases in conductance occur in much the same time (*3*), the effective transmitter equilibrium potential is −10 to −20 mv (*1*, *2*).

One implication of some significance which may be derived from these results is that different excitatory neurotransmitters in the central nervous system may have different effects on ionic conductances in postsynaptic membranes. A transmitter which increased only sodium conductance in a postsynaptic membrane would be more effective in synaptic transmission than one which increased sodium and potassium conductance equally, that is, a quantum of transmitter which increased only sodium conductance would produce more depolarization than would be obtained if both sodium and potassium conductances were increased by about the same amount as at cholinergic neuromuscular junctions. The finding of the rather selective effect on sodium conductance of the transmitter at the squid giant synapse is of particular interest in connection with the hypothesis that the sodium and potassium channels may be separately activated by transmitters (*3*).

PETER W. GAGE
School of Physiology, University of New South Wales, Kensington, N.S.W., Australia
JOHN W. MOORE
Department of Physiology and Pharmacology, Duke University, Durham, North Carolina, and *Marine Biological Laboratory, Woods Hole, Massachusetts*

References and Notes

1. P. Fatt and B. Katz, *J. Physiol.* (*London*) **115**, 320 (1951).
2. A. Takeuchi and N. Takeuchi, *J. Neurophysiol.* **22**, 395 (1959); *J. Physiol.* (*London*) **154**, 52 (1960).
3. P. W. Gage and C. M. Armstrong, *Nature* **218**, 363 (1968).
4. R. Miledi, *J. Physiol.* (*London*) **192**, 379 (1967); J. S. Kelly and P. W. Gage, unpublished observations.
5. T. H. Bullock, *J. Neurophysiol.* **11**, 343 (1948).
6. In addition to the excitatory synaptic transmission between giant pre- and postsynaptic axons, there are additional excitatory pathways provided by small fibers in the presynaptic nerve trunk which make synaptic contact near the soma of the postsynaptic axon [J. Z. Young, *Phil. Trans. Roy. Soc. London Ser. B* **229**, 465 (1939); S. H. Bryant, *J. Gen. Physiol.* **42**, 609 (1959); R. Miledi and C. R. Slater, *J. Physiol.* (*London*) **184**, 473 (1966)]. Preliminary experiments showed a number of complications if the "accessory pre-fibers" were stimulated also.
7. S. Hagiwara and I. Tasaki, *J. Physiol.* (*London*) **143**, 114 (1958); A. Takeuchi and N. Takeuchi, *J. Gen. Physiol.* **45**, 1181 (1962).
8. J. Del Castillo and B. Katz, *J. Physiol.* (*London*) **125**, 546 (1954); *Progr. Biophys.* **6**, 121 (1956).
9. J. W. Moore and W. J. Adelman, Jr., *J. Gen. Physiol.* **45**, 77 (1961).
10. Supported by NIH grant NB 03437.

2 June 1969; revised 25 July 1969

Thyrocalcitonin: Evidence for Physiological Function

Abstract. *A calcium salt given by stomach tube in modest amounts, such as might be ingested in a normal meal, produced hypercalcemia in acutely thyroidectomized fasted rats, whereas in rats with intact thyroid glands the same dose of calcium had little or no detectable effect, presumably because of release of thyrocalcitonin. Thyrocalcitonin apparently protects against hypercalcemia during feeding after deprivation.*

The mammalian thyroid gland, by virtue of its ability to release the hypocalcemic polypeptide, thyrocalcitonin (*1*), can protect against hypercalcemia produced in the laboratory by artificial procedures, such as parenteral administration of a calcium salt (*2*), injection of parathyroid hormone (*3*), or treatment with massive doses of vitamin D (*4*). Nevertheless, there has been justified skepticism concerning the physiological significance of thyrocalcitonin, because the experimental evidence for protection against hypercalcemia by the thyroid gland has been based entirely on results obtained under circumstances never encountered in normal life. Furthermore, the experiments of Bronner *et al.* (*5*) had indicated that even at high rates of calcium absorption from the gut, the concentrations of calcium in the serum of normal and thyroidectomized rats did not differ significantly. We now report our results on the feeding (by stomach tube) after fasting of ordinary amounts of calcium to thyroidectomized animals and animals with intact thyroid glands. In related experiments two other groups obtained ambiguous results (*6*).

A modest dose of calcium intragastrically produced marked hypercalcemia persisting at least 2 hours after the gavage in thyroidectomized rats, whereas the increase in the concentration of calcium in the serum in sham-operated rats was minimal and short-lived (Fig. 1). In these young male albino rats the parathyroid glands had been transplanted to the cervical strap muscles at 48 days of age. One week later the concentration of calcium in the serum was analyzed after the rats were fasted overnight, with 9 mg/100 ml or more serving as an indication that the transplanted parathyroid glands were functional. Rats meeting this criterion were divided randomly into four groups on the following day, and food (Purina laboratory chow) was withheld for 24 hours. Some of the rats were thyroidectomized by blunt dissection (without cautery) under ether anesthesia and others underwent sham operation. Calcium (15 mg per 100 grams of body weight; as a 1 percent solution of calcium chloride) was given by stomach tube immediately after the operation. Control rats, some thyroidectomized and some with thyroid gland intact, were given an equal volume of 0.9 percent sodium chloride by the same route. Blood samples for calcium analysis were obtained by cardiac puncture at 60 minutes and 120 minutes after gavage. The concentration of calcium in serum was determined in the Technicon Autoanalyzer (*7*) within 2 hours after the blood was collected. Since the concentrations of calcium in the serum of thyroidectomized rats and normal rats given sodium chloride were not significantly different, they are presented as combined means in Fig. 1, and represent the control situation.

Similar experiments were conducted with acutely thyroparathyroidectomized rats (without cautery), because a separate experiment (not shown) demonstrated that removal of the parathyroid glands did not impair the ability of the thyroid gland to protect against hypercalcemia after oral ingestion of a calcium load. In 13 experiments involving 280 rats the amounts of calcium admin-

istered varied from 1 to 20 mg per 100 grams of body weight. In every instance the concentration of calcium in serum was significantly higher 60 minutes after calcium gavage in thyroparathyroidectomized rats than in sham-operated rats. The intact thyroid gland protected completely against hypercalcemia after as much as 8 mg of calcium per 100 grams of body weight by gavage, while in the thyroparathyroidectomized rat as little as 1 mg of calcium per 100 grams of body weight produced a significant increase in the concentration of calcium in serum (Table 1).

The effect of voluntary consumption of food on the concentration of calcium in serum in the presence and absence of the thyroid gland also was determined. In an initial experiment, fasted rats trained to consume their entire day's ration in 60 minutes were thyroparathyroidectomized or sham-operated and then given access to a 1 percent calcium diet for 90 minutes. The average intake of calcium during this period was 55 mg. At the end of the 90 minutes the concentration of calcium in serum of the thyroparathyroidectomized rats was 11.8 ± 0.3 mg/100 ml; that of the sham-operated rats was 10.4 ± 0.3 mg/100 ml ($P < .01$). The rise in concentration of calcium in serum in the rats deficient in thyrocalcitonin, although substantial, was less than when smaller amounts of calcium were given as calcium chloride by gavage (Fig. 1 and Table 1). Presumably, other constituents of the diet tend to impede and retard the absorption of calcium when it is fed as part of a mixed ration.

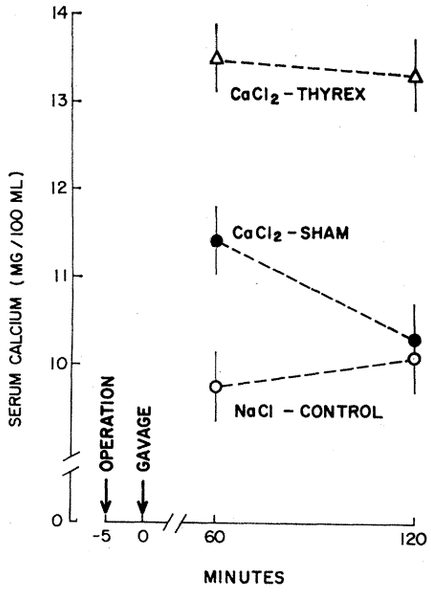

Fig. 1. Hypercalcemia after calcium administration (15 mg per 100 grams of body weight as $CaCl_2$) by gavage in thyroidectomized fasted rats with parathyroid transplants. Extent and duration of hypercalcemia is greatly reduced in rats with thyroid intact. There were five rats in each group given calcium. Two of the four control rats (NaCl) were thyroidectomized and two were sham-operated (body weight, 250 to 300 grams). The vertical lines represent the standard errors. Difference between sham-operated and thyroidectomized rats given calcium was significant at $P < .01$.

The thyroid gland, presumably through thyrocalcitonin, protects rats against hypercalcemia when moderate amounts of calcium are ingested rapidly, as likely occurs in natural life when a period of starvation or food restriction is followed by an episode of abundance. Whether or not the thyroid gland also protects against hypercalcemia during a normal meal that follows a "fast" of only a few hours has not yet been determined.

Because of the slow development of deficiency of thyroxine and triiodothyronine after thyroidectomy and the very short interval between surgery and blood collection in the experiments described, hypothyroidism in the classic sense could not have been a factor in the results observed.

We hypothesize that when ingested calcium is absorbed rapidly from the gastrointestinal tract in animals with intact thyroid glands the concentration of calcium in the blood rises, stimulating release of increasing amounts of thyrocalcitonin; the thyrocalcitonin brings the concentration of calcium in the blood back to or toward normal by inhibiting bone resorption (*8*) and possibly also by stimulating bone accretion (*9*). To explain the results in Table 1, indicating that in rats with intact thyroid glands there was no increase in the concentration of calcium in blood at low levels of calcium intake, we suggest that the thyroid gland is so sensitive to the concentration of calcium in blood that it responds rapidly to increases too small to be detected with certainty by our analytical procedure (*10*). Radioimmunoassay of the concentration of thyrocalcitonin in blood (*11*) after oral administration of small amounts of calcium should help to settle this question concerning the thyrocalcitonin response of the thyroid gland to a minute elevation in blood calcium.

We conclude that the physiological function of thyrocalcitonin is to protect against hypercalcemia during rapid absorption of calcium from the gastrointestinal tract. By so doing, thyrocalcitonin may contribute to the overall conservation of calcium for the skeleton by minimizing losses of calcium from the blood into the urine and the gastrointestinal tract and would tend to prevent calcification of soft tissues, particularly nephrocalcinosis, that otherwise might result from repeated bouts of hypercalcemia.

Table 1. Concentration of calcium in the serum of rats with thyroid intact (sham-operated) and of thyroparathyroidectomized (TPTX) rats 60 minutes after operation and intragastric administration of calcium chloride. The rats were 37 days old, weighed 120 to 140 grams, and had been fasted overnight. There were four animals in each group. The difference between sham-operated and TPTX rats, all groups combined, is significant at $P < .001$.

Intragastric calcium (mg/100 g)	Serum calcium (mg/100 ml)		P
	Sham-operated*	TPTX*	
1	10.60	11.40	<.05
2	10.42	11.40	<.005
4	10.70	12.12	<.005
8	10.92	13.52	<.001

* Standard error, ± 0.26.

T. KENNEY GRAY
PAUL L. MUNSON
Department of Pharmacology, School of Medicine, University of North Carolina, Chapel Hill 27514

References and Notes

1. P. F. Hirsch and P. L. Munson, *Physiol. Rev.* **49**, 548 (1969).
2. R. V. Talmage, J. Neuenschwander, L. Kraintz, *Endocrinology* **76**, 103 (1965).
3. A. H. Tashjian, Jr., *ibid.* **78**, 1144 (1966); P. F. Hirsch and P. L. Munson, *ibid.* **79**, 655 (1966).
4. H. F. DeLuca, H. Morii, M. J. Melancon, Jr., in *Parathyroid Hormone and Thyrocalcitonin (Calcitonin)*, R. V. Talmage and R. F. Bélanger, Eds. (Excerpta Medica, Amsterdam, 1968), p. 448.
5. F. Bronner, P. J. Sammon, R. E. Stacey, B. G. Shah, *Biochem. Med.* **1**, 261 (1967).
6. C. E. Lowe, E. D. Bird, W C. Thomas, Jr., *Endocrinology* **22**, 261 (1962); J. Lederer, F. Stein, A. M. Arnould, *Ann. Endocrinol.* **30**, 132 (1969).
7. H. J. Gitelman, *Anal. Biochem.* **18**, 521 (1967).
8. G. Milhaud, A. M. Perault, M. S. Moukhtar, *C. R. Hebd. Seances Acad. Sci. Paris* **261**, 813 (1965); J. Friedman and L. R. Raisz, *Science* **150**, 1465 (19[illegible] M. A. Aliapoulios, P. Goldhaber, P. L. Munson, *ibid.* **151**, 330 (1966).
9. P. F. Hirsch, H. Orimo, A. Sliwowski, *Fed. Proc.* **28**, 383 (1969).
10. C. W. Cooper, P. F. Hirsch, P. L. Munson, in preparation.
11. M. R. Lee, L. J. Deftos, J. T. Potts, Jr., *Endocrinology* **84**, 36 (1969).
12. Supported by PHS grant AM 10558, PHS general research support grant FR 5406, and PHS special research fellowship (T.K.G.). We thank R. T. James for technical assistance.

20 May 1969; revised 10 July 1969