much as 80 mv for 50 msec, as recorded with a second intracellular electrode) never caused a spike in the main LG axon, nor was there evidence for electrically excitable activity in the soma membrane (Fig. 2D). This result indicates that the electrotonic spread of soma depolarization is insufficient to activate axonal spike-initiating sites, and would be predicted from the length and small diameter of the neurite.

After several experiments, injection of dye into the axon was used to identify the recorded soma as that of LG. Cells showing large soma potentials tended to fill more rapidly than those showing small ones, suggesting that the length and diameter of the neurite is variable. Longer, thinner neurites presumably provide an extra impediment to the flow of dye, and also impose more electrotonic decrement.

In the thinness of its neurite and the heterolateral position of its soma the LG resembles the nonseptate median giants, whose architecture in the brain has recently been elucidated with conventional anatomical techniques (see *10*).

The detailed morphology of an identified interneuron has thus been worked out with a new and useful anatomical technique. The results show the LG to be a conventional neuron with a single soma which is electrically and spatially isolated from the conductile process, and whose membrane is electrically inexcitable. Previous microelectrode explorations of crustacean segmental ganglia may have failed to reveal soma responses of other interneurons because they, too, are more isolated from conductile and integrative sites than are the somata of motoneurons.

M. REMLER
A. SELVERSTON
D. KENNEDY

Department of Biological Sciences,
Stanford University,
Stanford, California 94305

References and Notes

1. C. A. G. Wiersma, *J. Neurophysiol.* **10**, 23 (1947); A. Watanabe and H. Grundfest, *J. Gen. Physiol.* **45**, 267 (1961).
2. G. E. Johnson, *J. Comp. Neurol.* **36**, 323 (1924); J. D. Robertson, *Ann. N.Y. Acad. Sci.* **94**, 339 (1961); K. Hama, *Anat. Rec.* **141**, 275 (1961).
3. C. A. G. Wiersma, *Cold Spring Harbor Symp. Quant. Biol.* **17**, 155 (1952).
4. E. J. Furshpan and D. D. Potter, *J. Neurophysiol.* **145**, 289 (1959).
5. D. Kennedy, in *Comparative Physiology and Biochemistry*, O. Lowenstein, Ed. (Academic Press, New York, 1966), vol. 2, p. 117.
6. K. Takeda and D. Kennedy, *J. Cell. Comp. Physiol.* **64**, 165 (1964).
7. E. A. Kravitz, A. O. W. Stretton, J. Alvarez, E. J. Furshpan, *Fed. Proc.* **27**, 749 (1968).
8. A. van Harreveld, *Proc. Soc. Exp. Biol. Med.* **34**, 428 (1936).
9. M. Otsuka, E. A. Kravitz, D. D. Potter, *J. Neurophysiol.* **30**, 725 (1967).
10. E. Horiuchi, H. Hayashi, I. Takahashi, *Nature* **212**, 831 (1966).
11. Supported by PHS grant NB-02944 and AFOSR grant AFOSR-334, and by a PHS postdoctoral fellowship to A.S. We thank Mrs. P. Hanawalt for technical assistance, and Dr. E. A. Kravitz and his collaborators for sharing their extensive experience with dyes, including the one used in these experiments. The Procion Yellow, M4RS, was a gift from Imperial Chemical Industries America, Inc., Stamford, Conn.

5 July 1968

Insulin Secretion from Toadfish Islet Tissue Stimulated by Pyridine Nucleotides

Abstract. *The addition of glucose stimulated release of insulin from the isolated islet tissue of the toadfish incubated in vitro. Reduced nicotinamide-adenine dinucleotide also stimulated insulin release, whereas the oxidized form had no effect. Both oxidized and reduced nicotinamide-adenine dinucleatide phosphate stimulated insulin release, but the reduced form was significantly more effective.*

Athough the role of glucose in stimulating insulin secretion from the beta cells of the islets of Langerhans has been well studied with the isolated perfused pancreas (*1, 2*) and slices of pancreas (*3–6*), the mechanism controlling the insulin release remains unknown. Several theories have been proposed (*7–9*), and the one most widely held is that glucose acts by giving rise, during metabolism, to some substrate or cofactor which then triggers insulin release (*9*). This is supported by the finding that glucose has no effect on insulin secretion when its metabolism is blocked by 2-deoxyglucose (*10*), glucosamine (*11*), mannoheptulose (*3, 4*), 2,4-dinitrophenol (*2, 3, 5*), or anoxia (*3, 5, 12*).

We have examined the effect of a number of metabolic intermediates and cofactors whose concentration in the islets might be expected to change as a result of glucose metabolism. We used the toadfish as the experimental animal. In contrast to mammals, the islet tissue of the toadfish is separated from the acinar pancreas and segregated into one or more discrete bodies; thus difficulties encountered by the presence of acinar tissue are eliminated. In our studies, reduced pyridine nucleotides markedly stimulated insulin release from islet tissue.

Toadfish islets (1 to 6 mg each) were obtained as described (*13*), decapsulated, and incubated for 10 minutes in 0.140*M* NaCl buffered at *p*H

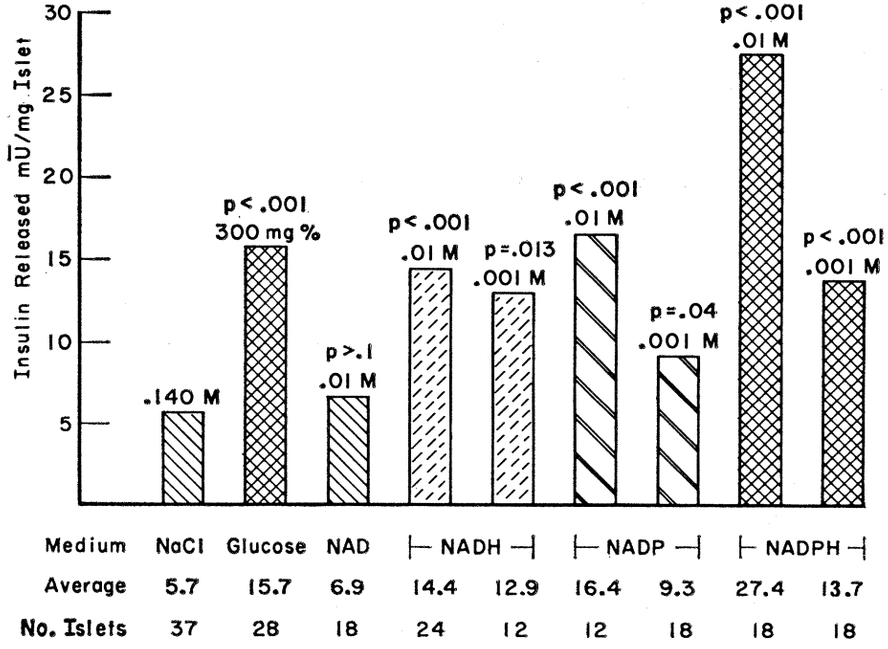

Fig. 1. Effect of pyridine nucleotides (NAD, NADH, NADP, NADPH) on insulin release from toadfish islets. The *P* values shown refer to the difference between the test substance indicated and the NaCl control.

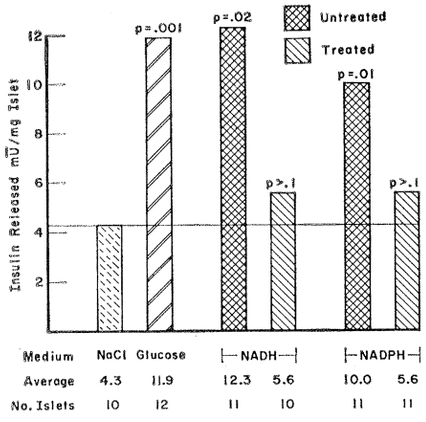

Fig. 2. Effect of destruction of reduced pyridine nucleotides on their ability to release insulin from toadfish islets. The concentration of the pyridine nucleotides was $1 \times 10^{-3}M$. The P values shown refer to the difference between the test substance indicated and the NaCl (0.140M) control. In the experiment with glucose its concentration was 300 mg per 100 ml of incubation medium.

7.0 with $1 \times 10^{-3}M$ phosphate buffer. This treatment removes insulin and other protein which may contaminate the surface of the islets. The islets were then rinsed in fresh medium of the same composition, transferred to tubes containing 100 μl of test medium and incubated for 60 minutes at room temperature (23° to 25°C). After this last incubation, the islets were removed with glass hooks, weighed, and discarded. The incubation medium was assayed for insulin-like activity (ILA) by the rat epididymal fat pad method (*14*); an immunological method was not used because of the poor cross-reactivity between toadfish insulin and antibody to mammalian insulin. The amount of activity in the medium was of such magnitude that the incubation medium was diluted 2000-fold for analysis. This activity can be neutralized by large amounts of antiserum to mammalian insulin.

The addition of 300 mg of glucose per 100 ml of incubation medium increased the amount of ILA which was released into the medium while the whole islets were incubated for 60 minutes at 25°C (Fig. 1). In contrast to these results, the same concentration of glucose had no significant effect on insulin secretion when the incubations were carried out at 0°C (data not shown). This finding is consistent with the theory that glucose must be metabolized in order to stimulate insulin secretion.

In order to test the effect of pyridine nucleotides on insulin release (Fig. 1), the nucleotides were dissolved in water, neutralized to pH 7.0, and diluted with an equal volume of 0.280M NaCl buffered at pH 7.0 with $2 \times 10^{-3}M$ phosphate buffer; all solutions were kept at 0°C until used. Whereas $10^{-2}M$ nicotinamide-adenine dinucleotide (NAD) had no effect on the amount of ILA released from whole islets, as little as $10^{-3}M$ reduced nicotinamide-adenine dinucleotide (NADH) produced a two-fold increase (Fig. 1). This is similar to that produced by 300 mg of glucose per 100 ml of incubation medium. In contrast to these results, at $10^{-2}M$ both the oxidized (NADP) and reduced (NADPH) forms of nicotinamide adenine dinucleotide phosphate stimulated insulin release; nevertheless the reduced form was significantly more effective ($P = .01$). At $10^{-3}M$ the effect of the oxidized form was of doubtful significance, whereas the effect of $10^{-3}M$ NADPH was highly significant. Lower concentrations of NADH and NADPH have not yet been tested. None of the pyridine nucleotides themselves showed any insulin-like activity.

In order to demonstrate that the effect of the reduced pyridine nucleotides was not due to some contaminant, control incubations were carried out with solutions in which these coenzymes were destroyed with acid (*15*). Destruction was confirmed by following the disappearance of the 340-nm peak in a spectrophotometer. After the reduced pyridine nucleotides were destroyed, the ability of these solutions to stimulate insulin release was virtually eliminated (Fig. 2).

Our results demonstrate that the pyridine nucleotides can trigger the release of insulin in vitro. Although the concentrations of the pyridine nucleotides used in these experiments are somewhat higher than the concentration calculated from the data of Lindall (*16*) to be present in islet tissue (approximately $2 \times 10^{-4}M$), they are of the same order of magnitude. Furthermore, it is not known how much of the pyridine nucleotide added in our experiments actually reached the site where it exerted its effect. Therefore it seems reasonable to postulate that the effects observed may be physiological, and that changes in the concentrations of the reduced pyridine nucleotides brought about by changes in blood glucose concentrations may play an important role in the physiological control of insulin secretion. Sulfhydryl groups have previously been reported to be important in maintaining the integrity of beta-cell membranes (*17*) and these observations suggest that the pyridine nucleotides could control the release of insulin by modifying the state of oxidation of these membrane sulfhydryl groups.

D. WATKINS
S. J. COOPERSTEIN
Department of Anatomy, University of Connecticut Schools of Medicine and Dental Medicine, Storrs 06268 and *Marine Biological Laboratory, Woods Hole, Massachusetts*
P. K. DIXIT
A. LAZAROW
Department of Anatomy, University of Minnesota School of Medicine, Minneapolis 55455, and *Marine Biological Laboratory, Woods Hole, Massachusetts*

References and Notes

1. E. Anderson and J. A. Long, *Endocrinology* **40**, 92 (1947); G. M. Grodsky, A. A. Batts, L. L. Bennett, C. Vcella, N. B. McWilliams, D. F. Smith, *Amer. J. Physiol.* **205**, 638 (1963).
2. G. M. Grodsky, L. Bennett, A. Batts, N. McWilliams, C. Vcella, *Fed. Proc.* **21**, 202 (1962).
3. H. G. Coore and P. J. Randle, *Biochem. J.* **93**, 66 (1964).
4. H. G. Coore, P. J. Randle, E. Simon, P. F. Kraicer, M. C. Shelesnyak, *Nature* **197**, 1264 (1963).
5. H. G. Coore and P. J. Randle, in *The Structure and Metabolism of the Pancreatic Islets*, S. E. Brolin, B. Hellman, H. Knutson, Eds. (Pergamon, New York, 1964), p. 295; H. G. Coore and P. J. Randle, *Biochem. J.* **84**, 78P (1962).
6. H. Frerichs, U. Reich, W. Creutzfeldt, *Klin. Wochenschr.* **43**, 136 (1965); P. Mialhe and V. Meyer, *Compt. Rend.* **253**, 1861 (1961); P. R. Bouman, *Acta Endocrinol.* **35**, 560 (1960); J. L. R. Candela, *Proc. Congr. Int. Diabetes Fed. 4th* **629** (1961).
7. J. R. Turtle, G. K. Littleton, D. M. Kipnis, *Nature* **213**, 727 (1967).
8. A. Lazarow, *Recent Progr. Hormone Res.* **19**, 489 (1963).
9. G. M. Grodsky and P. H. Forsham, *Ann. Rev. Physiol.* **28**, 347 (1966).
10. C. Kilo, C. L. Long, Jr., R. M. Bailey, M. B. Koch, L. Recant, *J. Clin. Invest.* **41**, 1372 (1962); J. L. R. Candela, D. Martin-Hernandez, T. Castilla-Cortazar, *Med. Exp. (Basel)* **11**, 47 (1964).
11. J. M. Martin and G. Bambers, *Fed. Proc.* **23**, 409 (1964).
12. G. M. Grodsky and H. Tarver, *Nature* **177**, 223 (1956).
13. A. Lazarow, S. J. Cooperstein, D. K. Bloomfield, C. T. Friz, *Biol. Bull.* **113**, 414 (1957).
14. A. E. Renold, D. B. Martin, Y. M. Dagenais, J. Steinke, R. J. Nickerson, M. C. Sheps, *J. Clin. Invest.* **39**, 1487 (1960).
15. F. Schlenk, in *The Enzymes*, J. B. Sumner and K. Myrback, Eds. (Academic Press, New York, 1951), vol. 2, part 1, p. 263.
16. A. W. Lindall, *Comp. Biochem. Physiol.* **22**, 297 (1967).
17. S. J. Cooperstein, D. Watkins, A. Lazarow, in *The Structure and Metabolism of the Pancreatic Islets*, S. E. Brolin, B. Hellman, H. Knutson, Eds. (Pergamon, New York, 1964), p. 389; D. Watkins, S. J. Cooperstein, A. Lazarow, *Anat. Rec.* **157**, 338 (1967).
18. Supported by grants AM-09059 to S.J.C. and AM-06517 to A.L. from National Institute of Arthritis and Metabolic Diseases. This research was carried out during the tenure of a Life Insurance Medical Research Fund fellowship awarded to D.W.

12 June 1968