cumulated in the peripheral and central zones, calcium and phosphorus x-ray measurements were conducted on an entire micro-mass culture or on the central and peripheral zones separately. This was done on micro-mass cultures prepared for scanning electron microscopy and coated with carbon. The results showed that 6-day-old cultures treated with phosphate from day 2 accumulated 10 to 15 times more calcium in the peripheral zones and 3 to 4 times more phosphorus. The Ca/P ratio of this mineral was 1.56. There was no significant difference in control cultures without added phosphate between the central and the peripheral zones and the whole cell layer.
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Calmodulin Activation of Adenylate Cyclase in Pancreatic Islets

Abstract. *Pancreatic islets contain calmodulin. The protein binds to a particulate fraction derived from the islets and stimulates adenylate cyclase activity in this subcellular fraction, both phenomena being activated by ionized calcium. A calcium-dependent stimulation of adenylate cyclase by endogenous calmodulin may contribute to the accumulation of adenosine 3',5'-monophosphate evoked by insulin releasing agents in the islet cells.*

Most insulin-releasing agents (secretagogues) examined for such a purpose increase the concentration of adenosine 3',5'-monophosphate (cyclic AMP) in pancreatic islet cells. This applies to secretagogues that are chemically unrelated and do not share a common primary site of action in the B cell such as glucose, α-ketoisocaproate, hypoglycemic sulfonylurea, glucagon, theophylline, barium, and the calcium ionophore A23187 (*1*). In certain cases the accumulation of cyclic AMP is known to be due to a primary activation of adenylate cyclase (for example, by glucagon) or inhibition of phosphodiesterase (for example, by theophylline). In other cases, however, the mechanism leading to cyclic AMP accumulation remains poorly understood. All the above-mentioned secretagogues affect the fluxes and concentration of calcium (or barium) in islet cells (*2*). It is here proposed that the concentration of ionized calcium in islet cells may control the activity of adenylate cyclase by way of the calcium-dependent regulatory protein calmodulin (*3*).

Pancreatic islets were isolated from fed female albino rats (*4*), and the endogenous calmodulin was assayed as described elsewhere (*5*). A group of 2000 islets was homogenized in 0.4 ml of a solution of ethylenebis(oxyethylenenitrilo) tetraacetic acid (EGTA; 0.1 m*M*), and portions (1, 5, and 25 μl) of this homogenate were used in the assay. For the measurement of adenylate cyclase activity, the isolated islets were homogenized in tris-HCl buffer (25 m*M*, *p*H 7.6) containing $MgCl_2$ (5.0 m*M*), EGTA (1.5 m*M*), and dithiothreitol (3.0 m*M*) in a ratio of 100 islets to 0.2 ml of buffer. After a first centrifugation for 1 minute at 100*g* to remove cell debris and intact cells, the homogenate was either used as such or centrifuged for 20 minutes at 27,000*g* and 4°C. The pellet was resuspended and centrifuged again two to three times in the same volume of the same tris-HCl buffer. All experiments were conducted during 20 minutes of incubation at 37°C in a final volume of 75 μl (*6*). The reaction mixture contained (final concentration) tris-HCl (25 m*M*, *p*H 7.6), $MgCl_2$ (5.0 m*M*), EGTA (0.3 m*M*), 3-isobutyl-1-methylxanthine (0.5 m*M*), dithiothreitol (0.6 m*M*), bovine albumin (0.5 mg/ml; bovine albumin, fraction V, Sigma), ^{32}P-labeled adenosine triphosphate ([α-^{32}P]ATP) (13 μ*M*; 20 μCi/ml), an ATP-regenerating system consisting of creatine phosphate (20 m*M*) and creatine kinase (0.8 mg/ml; E.C. 2.7.3.2), and 15 μl of either the islet homogenate (4.6 ± 0.1 μg of protein) or particulate fraction derived from it (1.9 ± 0.1 μg of protein). The reaction mixture also contained, as required, $CaCl_2$ (up to 5.0 m*M*), guanosine triphosphate (GTP; 10.0 μ*M*), unlabeled calmodulin (up to 7 μ*M*), and NaF (10.0 m*M*). The enzymatic reaction was initiated by addition of the islet homogenate or particulate fraction, and terminated as described in (*7*). The separation of cyclic AMP from ATP was achieved by the procedure of Salomon *et al.* (*8*) with the use of Dowex 50 W-X4 and neutral alumina columns. For the measurement of ^{125}I-labeled calmodulin binding, the particulate fraction was added (time zero) to the same reaction mixture as that used in the assay of adenylate cyclase activity, except for the absence of the tracer amount of [α-^{32}P]ATP and presence of ^{125}I-labeled calmodulin (16 n*M*). After 20 minutes of incubation at 37°C, the binding reaction was terminated as described elsewhere. The separation of bound from free ^{125}I-labeled calmodulin was performed by immediate filtration of the diluted reaction mixture through EHWP Millipore filters (*9*). The labeled and unlabeled calmodulin were prepared from bovine pancreas as described (*5*, *9*). All results are expressed per milligram protein and presented as the mean (± standard error). Protein was determined by the method of Lowry *et al.* (*10*), bovine albumin being used as a standard.

The islets were found to contain calmodulin at a concentration of 0.13 pmole per islet, that is, about 0.2 pmole per microgram of protein or, assuming equal distribution in the intracellular water space (2 to 3 nl per islet), approximately 50 μ*M*. The latter concentration is somewhat higher than that reported (1 to 20 μ*M*) in other tissues (*3*).

The binding of ^{125}I-labeled calmodulin (16 n*M*) to a membrane-rich particulate fraction prepared from the islet homogenate was measured in the presence and absence of unlabeled calmodulin (7 μ*M*) to correct for nonspecific binding (*11*). The influence of the concentration of ionized calcium on the specific binding of ^{125}I-labeled calmodulin was investigated by adding $CaCl_2$ (0.05 to 4.0 m*M*) to the reaction mixture containing 0.3 m*M* EGTA. Relatively little labeled calmodulin was bound when small amounts of $CaCl_2$ were added (0.1 m*M* or less). A tenfold increase in binding was observed when the amount of added $CaCl_2$ was raised to 0.4 m*M* (Fig. 1A). Higher concentrations of $CaCl_2$ did not further enhance the binding of labeled calmodulin (*12*).

The effect of bovine pancreatic calmodulin (7 μ*M*) on the activity of adenylate cyclase was judged from paired measurements performed at different calcium concentrations in the absence or presence of the protein (*13*). To minimize the contamination by endogenous calmodulin and GTP, we performed these

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experiments with the particulate fraction as distinct from total islet homogenate. In the absence of exogenous calmodulin, $CaCl_2$, when added in concentrations exceeding 0.3 mM, caused a dose-related inhibition of adenylate cyclase activity in the particulate fraction (Fig. 2). A comparable dose-related inhibition was the predominant effect of $CaCl_2$ on the activity of adenylate cyclase in the total islet homogenate (Table 1), as previously reported (*14*).

As long as the amount of added $CaCl_2$ did not exceed 0.2 mM, calmodulin failed to affect the rate of cyclic AMP formation by the particulate fraction (Fig. 1B). A significant stimulatory effect of calmodulin occurred at higher concentrations of $CaCl_2$. Regression analysis of the individual data (N = 64) obtained when 0.2 to 0.5 mM $CaCl_2$ was added to the EGTA-containing reaction mixture indicated a highly significant correlation ($P < .001$) between the magnitude of the calmodulin-induced increment in adenylate cyclase activity and the concentration of added $CaCl_2$, with a threshold value at a calculated concentration of ionized Ca^{2+} (*15*) close to $10^{-7}M$ and an apparent K_m (Michaelis constant) close to $10^{-5}M$. Thus, the range of concentrations in which a dose-related response occurred was compatible with current values for the cytosolic concentration of ionized Ca^{2+} (*16*). The calmodulin-induced increment in adenylate cyclase activity was most marked when 0.5 mM $CaCl_2$ was added to the reaction mixture. Although the mean absolute value for such an increment was decreased by increasing the $CaCl_2$ concentration from 0.5 to 1.0 mM, its value relative to the paired reading in the absence of calmodulin was little affected at the high $CaCl_2$ concentration, averaging 65 and 53 percent with 0.5 mM and 1.0 mM $CaCl_2$, respectively. The stimulatory effect of calmodulin on adenylate cyclase was small. At the optimal $CaCl_2$ concentration (0.5 mM), the calmodulin-induced increment in reaction velocity (0.66 ± 0.14 pmole of cyclic AMP formed per milligram of protein and per minute) represented no more than 10 percent of the maximum velocity in the presence of NaF (10 mM).

Table 1. Effect of $CaCl_2$, glucose, and NaF on the activity of adenylate cyclase in pancreatic islet homogenates and a particulate fraction derived from the homogenate. The effect of each addition and its statistical significance were calculated from paired comparisons within the same group of experiments. Numbers in parentheses indicate numbers of experiments.

Addition	Concentration (mM)	Adenylate cyclase activity (pmole/mg-min)
Pancreatic islet homogenate		
No addition		1.70 ± 0.23 (9)
$CaCl_2$	0.05 to 0.2	1.58 ± 0.05* (21)
$CaCl_2$	0.5	1.25 ± 0.08† (10)
$CaCl_2$	1.0	0.98 ± 0.13† (5)
$CaCl_2$	5.0	0.31 ± 0.04† (3)
Glucose	16.7	1.65 ± 0.12* (3)
2-Deoxyglucose	16.7	1.58 ± 0.04* (2)
NaF	10.0	6.45 ± 0.96† (9)
Particulate fraction		
No addition		1.21 ± 0.21 (7)
NaF	10.0	7.17 ± 0.50‡ (7)

*Not significant. †$P < .001$ when compared with control (no addition) homogenate. ‡$P < .001$ when compared with control particulate fraction.

The calcium-dependency shown in Fig. 1B was characteristic of the calmodulin effect. When GTP (10 μM) was used to activate adenylate cyclase, a significant effect was present both in the absence and presence of $CaCl_2$. The relative magnitude of the GTP-induced increment was little affected by $CaCl_2$ and, over the whole range of calcium concentrations, averaged 89.5 ± 8.5 percent of the paired control value (Fig. 2). The nucleotide GTP slightly enhanced the stimulatory effect of calmodulin on adenylate cyclase. Thus, at high $CaCl_2$ concentrations (0.5 to 1.0 mM) and in the presence of GTP, the calmodulin-induced increment in velocity above the paired value found in the sole presence of GTP averaged 142.8 ± 20.3 percent (N = 6) of the appropriate control increment attributable to calmodulin in the absence of GTP at the same $CaCl_2$ concentration.

A calcium-dependent stimulation of adenylate cyclase by way of calmodulin in the intact B cell would account for a number of unexplained observations. First, it may explain the finding that a variety of chemically unrelated secretagogues, all known to cause calcium accumulation in the islet cells, increase the concentration of cyclic AMP in islet cells. It is unlikely that glucose activates adenylate cyclase directly (Table 1), although such activation was proposed elsewhere (*17*). Second, in intact islets not exposed to phosphodiesterase inhibitors, the glucose-induced accumulation of cyclic AMP is relatively modest and is abolished when the islets are deprived of extracellular calcium (*18*). Our findings indicate that the effect of calmodulin on islet adenylate cyclase is indeed modest and dependent on the concentration of Ca^{2+}.

We have previously emphasized the idea that, in the process of insulin release evoked by nutrient secretagogues, endogenous cyclic AMP acts as a potentiating rather than initiating factor, the effect

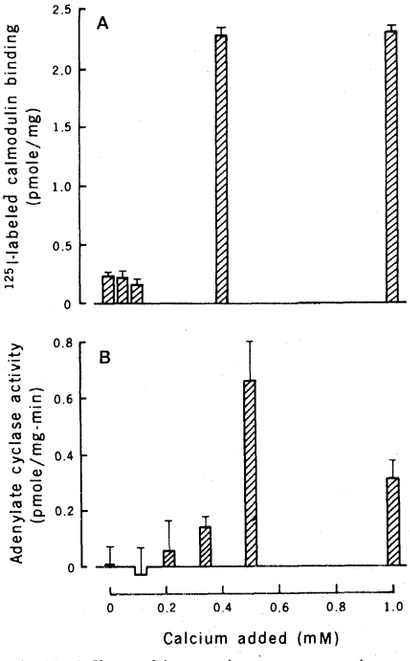

Fig. 1. Effect of increasing concentrations of $CaCl_2$ in a medium containing 0.3 mM EGTA on (A) the binding of ^{125}I-labeled calmodulin to a particulate fraction derived from pancreatic islets and (B) the magnitude of the calmodulin-induced increment in adenylate cyclase activity of the same particulate fraction. In (A) each mean value is derived from three to six measurements; in (B) each mean value is derived from 18 to 43 paired measurements. The control values for the synthesis of cyclic AMP in the absence of calmodulin are shown in Fig. 2.

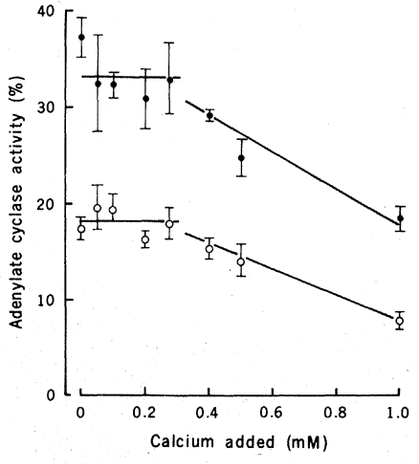

Fig. 2. Effect of increasing concentrations of $CaCl_2$ in a medium containing 0.3 mM EGTA on the activity of adenylate cyclase in an islet particulate fraction examined in the absence (open circles) or presence (closed circles) of GTP (10.0 μM). All results are expressed relative to the paired value for NaF-activated adenylate cyclase activity, which averaged 7.17 ± 0.50 pmole of cyclic AMP per milligram per minute (see Table 1). Each point is derived from 11 ± 2 measurements.

of glucose on calcium handling by the islet cells being the fundamental mechanism for stimulation of insulin release (*19*). The present study suggests that the effect of glucose on cyclic AMP synthesis may be secondary to its effect on the intracellular concentration of calcium. Since cyclic AMP itself facilitates insulin release, in part at least by causing an intracellular redistribution of calcium (*2*), the interaction between calcium, calmodulin, adenylate cyclase, and cyclic AMP in islets exposed to glucose would be well suited for amplification of the secretory response.

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12. The specific binding of ^{125}I-labeled calmodulin with 4.0 m*M* $CaCl_2$ (2.76 ± 0.25 pmole/mg) was not significantly different from that found with 1.0 m*M* $CaCl_2$ (2.30 ± 0.06 pmole/mg).
13. With 0.5 m*M* $CaCl_2$ a significant stimulation of adenylate cyclase activity was also observed when the calmodulin concentration was 0.3 μM.
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Cerebellar Plasticity: Modification of Purkinje Cell Structure by Differential Rearing in Monkeys

Abstract. *Dendritic branching in Purkinje and granule cells and the diameters of Purkinje cell somas were compared in several cerebellar areas of monkeys reared in isolation, with social experience, or in a large colony. In the colony-reared monkeys, spiny branchlets of Purkinje cells were more extensive in the paraflocculus and the nodulus than they were in the other two groups. Granule cell dendritic branching in the paraflocculus and nodulus did not differ across groups. In addition, Purkinje cell somas were larger in the uvula and the nodulus of the colony animals than in the other groups. These data indicate that the social and physical environment during development influences the morphology of cerebellar Purkinje cells.*

The cerebellum, a brain structure involved in coordination of movements, is vulnerable to postnatal trauma such as malnutrition (*1*) and x-irradiation (*2*), yet little, if any, evidence indicates that postnatal behavioral experience can modify cerebellar organization. According to a number of theoretical positions, cerebellar plastic changes should occur. For example, neural models of cerebellar functioning, such as that of Marr (*3*), predict that changes in the strength of cerebellar circuitry underlie the storage of learned motor sequences. Postnatal acquisition of skills requiring motor coordination is a continuing process, as any adult who has learned to drive a car can attest. Since the cerebellum plays a role in the execution of these learned skills, and since changes in cerebellar neuronal function correspond to the acquisition of a motor skill (*4*), the fine structure of the cerebellum may reflect the extent to which the rearing environment allows motor skills to develop.

Behavioral theorists have also argued for the effects of experience on the cerebellum. In particular, Prescott has suggested that abnormal cerebellar development, resulting from restricted social and maternal experience, might underlie aspects of the "primate isolation syndrome" (*5*). The syndrome, which is seen in monkeys after prolonged periods of isolation early in life, consists of a variety of abnormal, autistic behaviors, including social and sexual dysfunctioning as well as movement disorders (*6*). Postulated abnormal cerebellar development associated with this syndrome could possibly reflect the involvement of the cerebellum in social-emotional behavior (*7*).

Numerous studies in rodents, cats, and primates have shown that the fine structure of various brain regions can be altered by experience in development. For example, the extent of neuronal dendrites and the number of spines, the postsynaptic elements of one type of synapse, can be affected by the type and amount of experience during development (*8*). In general, these changes have been demonstrated primarily in forebrain structures such as the hippocampus and neocortex. Such anatomical changes may be involved in or mediate behavioral effects of experience. To date, similar studies have not been performed on hindbrain cortical structures such as the cerebellum. To examine possible anatomical plasticity in the cerebellum, the morphology of two cerebellar cell types was compared in monkeys reared under different conditions. We now report evidence for experience-dependent plasticity in the anatomy of cerebellar neurons (*9*).

Sixteen monkeys, *Macaca fascicularis*, were semirandomly assigned (*10*) to and reared for the first 6 months of life under one of three conditions. Six monkeys, three males and three females, reared in isolation (I) had a very limited sensory and motor environment, having been enclosed in a 1-m^3 Plexiglas cube contained within a sound-attenuating vault (*11*). The I monkeys neither saw nor had physical contact with another monkey during rearing, and there was little in the cage to encourage manipulation or play (*11*). Six monkeys, five males and one female, reared under social conditions (S) were housed in wire cages also about 1 m^3 in size. Cages for pairs of monkeys were adjacent, and 4 hours of play were allowed each day be-

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