understanding of cell-hormone interaction (*14*). Without cells in culture it would have been difficult to establish that the expression of a cell surface protein can be controlled by EGF, and we can now investigate the number of events that precede the appearance of LETS protein networks when EGF has bound to the cell surface. Since LETS protein is a differentiated product of fibroblasts, myoblasts, and some epithelial cells (*15*), the present findings support the notion that probably some of the differentiated functions of a given cell type are controlled by hormones. It will be of interest to investigate whether the production of collagen, glycosaminoglycan, and other differentiated products of fibroblasts are also controlled by EGF.

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Dopamine and Adenosine 3′,5′-Monophosphate Responses of Single Mammalian Sympathetic Neurons

Abstract. *Acetylcholine (ACh), dopamine, and dibutyryl-adenosine 3′,5′-monophosphate (dbcAMP) were applied iontophoretically to the rabbit superior cervical ganglion cells from triple-barreled micropipettes, and the response was recorded by intracellular techniques. All ganglion cells tested responded to the depolarizing action of ACh, whereas less than half of the cells that responded to ACh were hyperpolarized by dopamine. This effect was blocked by low concentrations of haloperidol. None of the cells examined responded to dbcAMP applied by iontophoresis. Hence, the present result is not consistent with the concept that a cyclic AMP mechanism underlies the hyperpolarizing effect of dopamine.*

Dopamine (DA) has been identified as the neurotransmitter, released on preganglionic stimulation from the small intensely fluorescent (SIF) cells, and responsible for the generation of the slow inhibitory postsynaptic potential (slow IPSP) of the rabbit superior cervical ganglion cell (*1*). It has been suggested that the action of DA depends on the intracellular cyclic adenosine 3′,5′-monophosphate (AMP) system that alters the membrane permeability by protein kinase–mediated phosphorylation; indeed, it was demonstrated that cyclic AMP is generated by presynaptic stimulation of, or by the application of DA to, the ganglion (*2, 3*). In support of this concept, it was reported that, as evaluated by the sucrose-gap method, monobutyryl AMP as well as the dibutyryl derivative (dbcAMP) caused a membrane hyperpolarization similar to that elicited by DA (*3*). However, results obtained in our laboratory with similar techniques failed to confirm these findings (*4*). Accordingly, our investigation was undertaken to evaluate, by microelectrode techniques, the effects of DA and dbcAMP on single sympathetic neurons. The intracellular recording techniques alleviate some of the shortcomings and ambiguity often associated with the sucrose-gap method, which records global activities of cell populations.

Acetylcholine (ACh), DA, and dbcAMP were applied by iontophoresis from triple-barreled micropipettes positioned close to the ganglion cells, and the response was recorded by intracellular techniques; a simplified experimental arrangement is illustrated in the inset of Fig. 1 (*5*). To exclude the possibility that the absence of responses of dbcAMP or DA (or both) could be due to excessive distance between the micropipette and the ganglion cell, the proximity of the micropipette was estimated in terms of the rise time of the ACh potential. In our study, only those cells that exhibited a rise time of ACh potentials of less than 30 msec were included; in many instances, neuronal spikes could be elicited by a strong current pulse delivered through the ACh micropipette.

The ACh potential was elicited by single current pulses of brief duration; the duration of current pulses varied from 5 to 10 msec in different experiments. Altogether, 31 cells (18 ganglia) showed a satisfactory ACh potential. The mean amplitude of ACh potentials was 8.7 ± 3.1 mv (mean ± S.D.), with a mean rise time of 27.9 ± 5.3 msec and a mean half-decay time of 46.0 ± 14.7 msec. The amplitude of ACh potentials remained relatively constant provided that the ejection current was unchanged. Of the 31 cells that exhibited a satisfactory ACh potential, 11 cells responded to iontophoresis of DA by a definite membrane hyperpolarization (Fig. 1A). In order to obtain DA responses, tetanic current pulses had to be used (30 hertz, 30 msec/pulse, for 4 to 5 seconds). The amplitude of DA hyperpolarization ranged from 0.5 to 4 mv, with a mean of 1.8 mv, and its duration varied from 4 to 12 seconds. Haloperidol (0.1 to 1 μM), a DA

antagonist, completely abolished the DA-induced membrane hyperpolarization in all 11 cells studied (Fig. 1B). The amplitude of ACh potentials was also slightly depressed by haloperidol at concentrations as low as 0.1 μM. However, the differential character of haloperidol action was apparent in that with some concentrations its anticholinergic action was minimal, whereas the block of DA effect was complete (Fig. 1B). The recovery of DA responses from the depressing action of haloperidol was slow and usually incomplete. Frequently, more than 30 minutes of washing was required for the DA response to attain 75 percent of its initial amplitude (Fig. 1C).

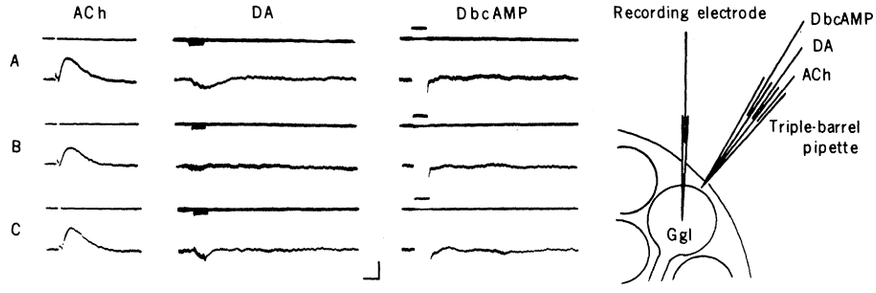

Fig. 1. The intracellular recording of the response of the rabbit superior cervical ganglion cell to iontophoretic applications of ACh, DA, and dbcAMP. (A) Control responses. (B) Responses after 10 minutes of haloperidol (0.1 μM) superfusion. (C) Responses after a 30-minute washing with Krebs solution. The upper tracing of each recording represents the current pulse. ACh potentials were elicited by a current pulse of 7 msec duration. The DA and dbcAMP responses were elicited by tetanic (30 hertz) current pulses of 30 msec duration. Records were taken from the same ganglion cell. Vertical bar of the calibration mark: 10 mv and 1×10^{-7} ampere for ACh potentials, 4 mv and 1×10^{-6} ampere for DA and dbcAMP responses. Horizontal bar of the calibration mark: 40 msec for ACh, 4 seconds for DA and dbcAMP responses. The diagram of the experimental arrangement is illustrated at the right.

The dbcAMP was also applied iontophoretically by means of tetanic current pulses. In order to select a suitable current duration, a preliminary study of the release of dbcAMP from the micropipettes was undertaken by a modification of Gilman's method (*6*). The current pulse of 5-second duration was optimal in that it consistently released the highest amount of dbcAMP from the micropipettes; the mean release of dbcAMP from three different micropipettes was estimated to be 56.13 pmole in 5 seconds of iontophoresis (30 hertz, 30 msec/pulse, 5×10^{-7} to 1×10^{-6} ampere). The responsiveness of each ganglion cell to dbcAMP was routinely examined with the use of current pulses of 10- to 30-second duration in addition to the pulses of 5 seconds. None of the 31 cells exhibited any detectable membrane response to iontophoresis of dbcAMP; negative results were obtained even when the ejection current was several times higher than that used to discharge DA (1×10^{-7} to 1×10^{-6} ampere and 2×10^{-8} to 4.6×10^{-7} ampere, respectively). Furthermore, in five experiments, superfusion of the ganglia for more than 30 minutes with a phosphodiesterase inhibitor (theophylline, 1 mM), did not affect the amplitude or time course of the DA hyperpolarization (three cells), nor did it unmask any membrane effect of dbcAMP (five cells).

Our data demonstrate that iontophoresis of DA to mammalian sympathetic neurons evoked a membrane hyperpolarization in many respects similar to the slow IPSP elicited by preganglionic nerve stimulation. The amplitude and duration of the DA-induced hyperpolarization were less than those of the slow IPSP (*7*). Obviously, the micropipette must be less intimately coupled with the surface of the cell membrane than the nerve endings of the SIF cell abutting the latter. Nevertheless, the rise time of ACh potentials (17 to 28 msec) obtained in our study was comparable with the rise time (4 to 10 msec) of the fast excitatory postsynaptic potentials (*8*), suggesting that the micropipette was reasonably close to the recording ganglion cell. Furthermore, the results obtained with haloperidol, which completely and differentially abolished the response of DA, establish that the DA-induced membrane hyperpolarization resulted from an action on specific postsynaptic dopaminergic receptors.

On the contrary, iontophoresis of dbcAMP did not elicit noticeable membrane effects in any of the 31 ganglion cells studied, including those exhibiting a DA hyperpolarization. These negative data could be due to the presence of ganglionic phosphodiesterase. However, prior treatment of the ganglion with theophylline did not unmask any hyperpolarizing effect of dbcAMP. Similarly, the apparent lack of responsiveness of the sympathetic neurons to iontophoresis of dbcAMP could be a result of insufficient release of dbcAMP from the micropipettes. The cyclic AMP content of the rabbit superior cervical ganglion appears to be very low, of the order of 20 pmole per milligram of protein (*2*); thus, only a very small amount of cyclic AMP is present in each ganglion cell. The amount of dbcAMP introduced by iontophoresis onto each ganglion cell appeared to be high compared to the cell content of cyclic AMP. It should also be emphasized that the dbcAMP used in our experiments should readily penetrate the interior of the cell membrane (*3*).

The important aspect of these experiments relates to the role of cyclic AMP in generating the dopaminergic slow IPSP. Our microelectrode data demonstrate that dbcAMP fails to duplicate the hyperpolarizing effect of DA in cells clearly exhibiting the latter. Hence, our result as well as those of others with respect to central neurons (*9*) suggest that further studies are needed to elucidate the role of cyclic nucleotides in synaptic transmission.

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6. The release of dbcAMP was measured from three different micropipettes containing 0.1M dbcAMP solution. The micropipette was placed in a small beaker containing 1 ml of 0.05M tris-HCl–0.04M EDTA buffer solution. Tetanic current pulses (30 hertz, 30 msec/pulse, 5×10^{-7} to 1×10^{-6} ampere) were used to discharge dbcAMP from the micropipette into the tris buffer solution; the duration of current pulses varied from 2 to 120 seconds. At the end of current

application, a 50-μl portion of the buffer solution was removed and assayed for cyclic AMP content by the method of A. G. Gilman [*Proc. Natl. Acad. Sci. U.S.A.* **67**, 305 (1970)], as modified by B. L. Brown *et al.* [*Biochem. J.* **121**, 561 (1971)].
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Artificial Pancreas Using Living Beta Cells: Effects on Glucose Homeostasis in Diabetic Rats

Abstract. *An artificial pancreas consisting of beta cells cultured on synthetic semipermeable hollow fibers was tested in rats with alloxan-induced diabetes. When implanted ex vivo as arteriovenous shunts in the circulatory system these devices lowered concentrations of plasma glucose from 533 to between 110 and 130 milligrams per 100 milliliters, increased concentrations of plasma insulin, and restored intravenous glucose tolerance tests essentially to normal.*

Transplantation of insulin-producing tissue constitutes a potentially important approach to the treatment of diabetics (*1*). Several studies have demonstrated that transplantation of the whole pancreas with vascular anastomosis in insulin-dependent diabetic patients restores glucose homeostasis to normal without further need for injection of exogenous insulin (*2*). Unfortunately, the morbidity and mortality associated with this procedure have been unacceptably high (*3*). Major difficulties have included (i) requirements for continuous immunosuppressive therapy after transplantation, (ii) problems associated with procurement of viable human donor pancreatic tissue, and (iii) the extensive surgery involved in implanting the donor pancreas, including the need to provide drainage for acinar enzymes. Although the use of isolated islets in place of the whole pancreas would simplify the surgical procedures (*4*), the problem of isolating large numbers of viable human islets from cadaver organs and the requirement for immunosuppressive therapy would still remain.

Various types of cells (*5*), including beta cells isolated from neonatal rat pancreas (*6*), have previously been successfully cultured for several weeks on the outside surfaces of bundles of synthetic capillaries perfused with tissue culture medium. Release of insulin into the perfusate, measured over 2-day intervals, remained relatively constant during this period and responded appropriately to changes in glucose concentration in the perfusion medium (*6*). More recent studies dealing with short-term insulin secretory dynamics showed that after an increase in the glucose concentration of the perfusate from 5.5 to 16.5 m*M* an increase in insulin levels in the effluent medium from the device could be detected within 5 minutes (*7*). The capillaries (Amicon XM-50 acrylic copolymer) used in these experiments had a nominal molecular weight cut-off of 50,000 and, therefore, were permeable to insulin but essentially impermeable to antibodies and lymphocytes. The walls of these fibers, therefore, act as a barrier against immune rejection, as is the case for chambers constructed from Millipore membranes that possess considerably larger pores (*8*).

The present experiments were undertaken to determine whether these devices would restore plasma glucose concentrations to normal in rats with allox-

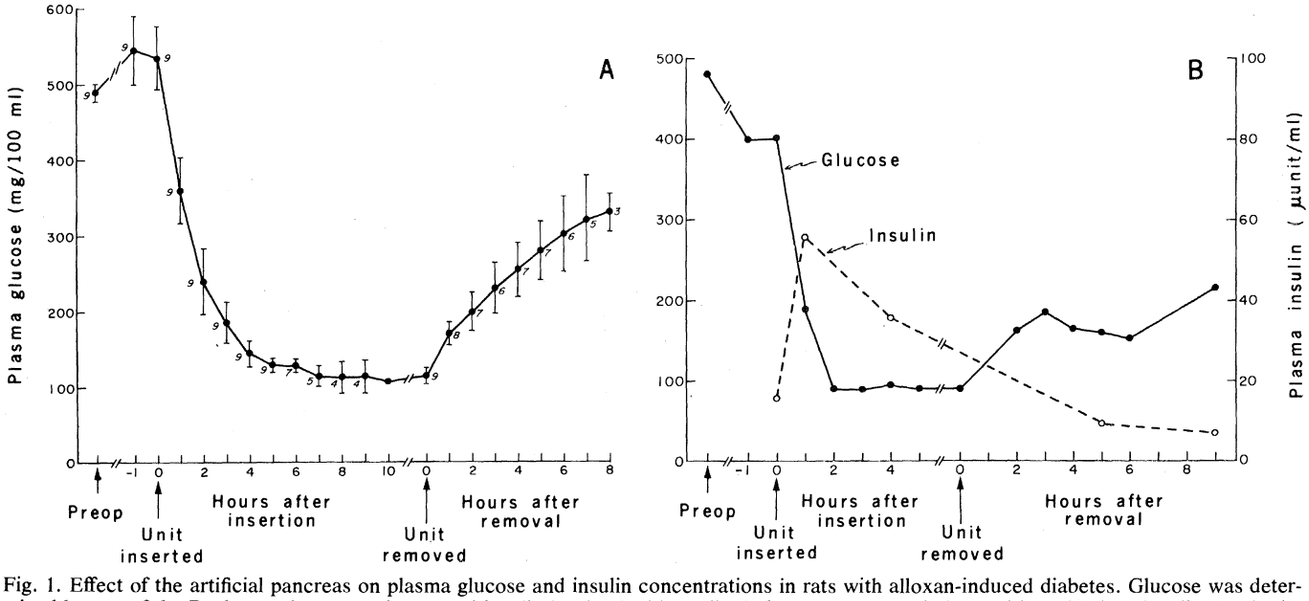

Fig. 1. Effect of the artificial pancreas on plasma glucose and insulin concentrations in rats with alloxan-induced diabetes. Glucose was determined by use of the Beckman glucose analyzer, and insulin by the double antibody immunoassay technique with purified rat insulin standards. Blood samples were obtained from the arterial side of the iliac artery-to-vein Silastic shunt. The preoperative sample was drawn prior to administration of anesthesia. After insertion of the iliac artery-to-vein shunt, samples were obtained at hourly intervals until concentrations of plasma glucose were relatively constant. The pancreatic device was then inserted into the shunt and left in place until the plasma glucose stabilized within the range of 100 to 150 mg per 100 ml. Devices were then removed and blood samples were again obtained at hourly intervals to follow the subsequent rise in plasma glucose and fall in circulating insulin levels. (A) Arterial plasma glucose levels (mean ± S.E.M.). The small numerals indicate the number of values used to compute each point. (B) Arterial plasma glucose and insulin concentrations in a representative diabetic animal. After insertion of the device in the shunt, there was a concomitant rise in plasma insulin and fall in plasma glucose. After the device was removed insulin levels fell while the plasma glucose rose.