able in practice, and a probe of the cloned Pst I cDNA fragment from pKD3 readily detects HDV RNA in serum of patients with acute or chronic HDV infections (*13*).

The nucleic acid hybridization technique with this cloned cDNA fragment as probe represents a useful noninvasive diagnostic assay for the identification of chronic HDV carriers who are at high risk of serious and progressive liver disease. The cDNA probe can be used for studying the replication of HDV in infected liver tissues, and synthetic oligonucleotides of the cDNA probe will be useful as cDNA primers.

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Differences in Adrenergic Recognition by Pancreatic A and B Cells

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The adrenergic control of glucose homeostasis is mediated in part through variations in the release of pancreatic hormones. In this study, purified pancreatic A and B cells were used to identify the recognition and messenger units involved in the adrenergic regulation of glucagon and insulin release. Catecholamines induced β-adrenergic receptor activity in A cells and α_2-adrenergic receptor activity in B cells. The two recognition units provoked opposite variations in the production of cellular cyclic adenosine monophosphate, the β-adrenergic unit enhancing the nucleotide's permissive effect on amino acid–induced glucagon release and the α_2-adrenergic unit inhibiting that upon glucose-induced insulin release. In both cell types, catecholamines interact powerfully with the synergistic control of hormone release by nutrient- and (neuro)hormone-driven messenger systems.

CATECHOLAMINES PLAY A MAJOR role in the rapid adjustment of various metabolic pathways to physiologic and pathologic demands (*1*). Their function is partly accomplished within the endocrine pancreas, where the hormonal secretion is under tight adrenergic control (*2–3*). The mechanisms whereby epinephrine and norepinephrine regulate insulin and glucagon release are still controversial (*4–5*), their investigation being hampered by the heterogeneous cell composition of the experimental models used so far. In vitro, the secretory response of unpurified islet tissue is markedly influenced by locally released hormones and other intercellular interactions (*6–8*), which stresses the need to investigate the regulation of pancreatic hormone release in purified islet cell preparations. This requirement is even more crucial in analyzing the effects of catecholamines, because these compounds may interact with various islet cell types (*2–5*) and because they are, in addition, stored in intact islet tissue (*9*). For these reasons, we examined the effects of epinephrine, norepinephrine, and adrenergic agonists and antagonists on purified islet A and B cells.

By using the technique of autofluorescence-activated cell sorting, we separated islet cells on the basis of their ability to vary the redox state of their flavin and pyridine nucleotides according to the extracellular glucose concentration (*10, 11*). Such isolated cell suspensions are more than 95 percent pure and vital (*11*) and can be used in vitro to study the stimulus-secretion coupling within pancreatic A and B cells (*7, 8, 12*). With this method, it was previously shown that both the glucagon and the insulin release process undergo a synarchic regulation by nutrient- and hormone-driven control units (*7, 8*). The hormone-induced message appeared to be mediated by adenosine 3′,5′-monophosphate (cAMP), which was attributed a permissive role in the nutrient regulation of pancreatic hormone release (*7, 8, 12*). We have now studied the sites where catecholamines influence the secretory process of islet A and B cells and hence regulate glucose homeostasis.

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The addition of epinephrine to purified A cells provoked a dose-dependent increase in both cellular cAMP content and nutrient-induced glucagon release (Fig. 1). Both effects were detected from $10^{-8}M$ on, reached maximal amplitudes between 10^{-7} and $10^{-6}M$, and were, at $10^{-7}M$, comparable to those measured at $10^{-7}M$ norepinephrine (Fig. 2). In purified B cells, both catecholamines lowered basal cAMP content by 46 ± 12 percent (mean ± SE, $n = 4$). This effect was not associated with a further reduction in the secretory activity of isolated B cells, as may have been expected from cells whose impaired secretory function had already been attributed to a cAMP deficiency in the absence of catecholamines (*7*). In the presence of $10^{-8}M$ glucagon, isolated B cells regained the cAMP levels and secretory activity of intact islet tissue (*7, 12*); under this condition epinephrine provoked a dose-dependent and parallel inhibition of both cAMP formation and glucose-induced insulin release (Fig. 1). These suppressive effects were detected from $10^{-9}M$ on and reached an 80 percent inhibition at $10^{-7}M$; norepinephrine at $10^{-7}M$ induced a 75 percent inhibition (Fig. 2).

Our data illustrate that catecholamines interact directly with both pancreatic A and B cells, exerting a stimulatory action on glucagon release without necessary participation of other hormones, while their inhibitory effect on insulin release was noted only in the presence of an adenylcyclase activator such as glucagon. Isolated islets, with their intercellular interactions and different adrenergic recognition units, can thus not be expected to provide accurate information concerning the adrenergic effects on a particular cell type. With the use of pure B cells, the adrenergic inhibition of insulin release could be attributed to a decreased cAMP production over the entire concentration range tested, which makes it unnecessary to postulate cAMP-dependent and -in-

dependent inhibitory mechanisms depending on the catecholamine concentration used (*5*). The adrenergic control of glucagon release also appeared to be mediated by cAMP. These results fit into the concept that cAMP determines the sensitivity of both the insulin and the glucagon release process in response to nutrient stimuli (*7, 8*) and indicate, in addition, a powerful adrenergic control of this permissive effect. The physiological relevance of these in vitro data can be derived from the observation that both pancreatic A and B cells are sensitive to catecholamine concentrations that may occur in plasma (*1, 13*) and in the vicinity of nerve terminals (*14*). Our results further indicate that the adrenergic control of pancreatic hormone release in vivo will not depend only on the adrenergic recognition by the islet cells, but also on the local concentrations of other regulators of cAMP metabolism and on the prevailing nutrient supply. Circulating pancreatic hormone levels are, in addition, determined by the adrenergic regulation of the blood supply to the endocrine pancreas (*15*).

The opposing effects of catecholamines on pancreatic A and B cells led us to compare the type of adrenergic recognition by both cell types. We measured the effects of α- and β-adrenergic agonists and antagonists on cAMP formation and hormone release. In pancreatic A cells, only the addition of the β-adrenergic agonist isoproterenol exerted a stimulatory effect, as did epinephrine and norepinephrine (Fig. 2a). That β-adrenergic receptors transmit the adrenergic control in pancreatic A cells is indicated by the finding that only the β blocker propranolol prevented the adrenergic effects on cAMP formation and hormone release in pancreatic A cells (Fig. 2b) ($P < 0.05$ versus control by paired t testing). However, the insulin-containing B cells appeared not equipped with β-adrenergic receptors, as is evident from the inability of isoproterenol or propranolol to affect cAMP metabolism and insulin release in pure B-cell preparations (Fig. 2, c and d). Only clonidine, an α_2 agonist, reduced the rate of cAMP formation and insulin release by islet B cells, thus mimicking the effects of epinephrine and norepinephrine (Fig. 2c). Alpha-2 receptors were found to be responsible for the adrenergic recognition by pancreatic B cells, since phentolamine and yohimbine, both α_2 antagonists, counteracted the inhibitory effects of epinephrine on these cells (Fig. 2d). The α_1-selective compounds methoxamine and prazosine were ineffective in both A and B cells (Fig. 2). It is evident from these results that pancreatic A and B cells are equipped with different adrenergic recognition units that transmit opposite variations in the cellular cAMP levels, and which are thus primarily responsible for the divergence in the rate of glucagon and insulin release that is known to occur in several physiological and pathological conditions (*16*). The identification of α_2 receptors on B cells and β receptors on A cells is compatible with previous data obtained in unpurified

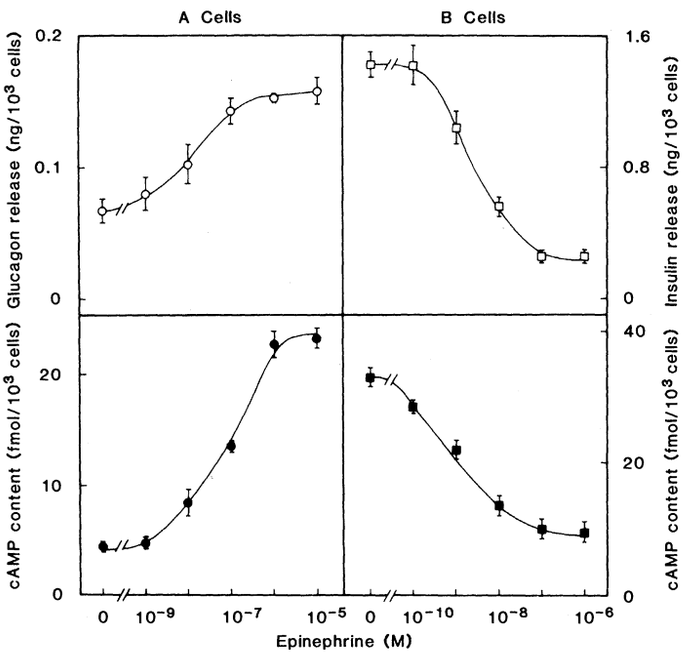
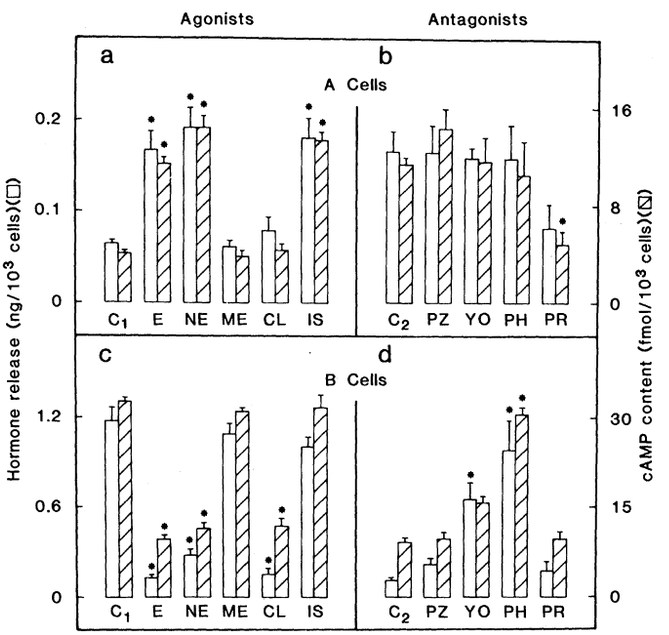


Fig. 1 (left). Dose-response curves for the effects of epinephrine on hormone release (○, □) and cAMP production (●, ■) by purified pancreatic A cells (○, ●) and B cells (□, ■). The cells were purified from male adult Wistar rats (*11*). After 20 hours of culture in CMRL-1066 supplemented with 10% (v/v) fetal calf serum, the cells were washed and finally suspended in Earle's-Hepes medium [*p*H 7.35; for composition see (*11*)] containing 1.4 m*M* glucose, 1 m*M* ascorbic acid, and 0.5% bovine serum albumin (Sigma) (EH). Samples of 5×10^4 cells were incubated for 15 minutes at 37°C in 750 μl of EH with or without epinephrine; this medium was supplemented with a mixture of alanine, arginine, and glutamine (2 m*M* each) for A cells, or with 20 m*M* glucose plus 10^{-8}*M* glucagon for B cells, in order to create the conditions where hormone release can be modulated by catecholamines (*7, 8*). During the last 5 minutes the cells were incubated with 3-isobutyl-1-methylxanthine (IBMX) in order to measure cAMP accumulation concomitantly with the amount of glucagon or insulin released. At the end of the 15-minute incubation period the cells were centrifuged and extracted for cAMP measurements (*12*), while the supernatant was analyzed for glucagon and insulin release (*11*). Glucagon and insulin release are expressed as nanograms



of hormone released per 15 minutes per 10^3 cells; the cellular cAMP content as femtomoles per 10^3 cells. Data express means ± SEM for four experiments. Fig. 2 (right). Effect of α- and β-adrenergic agonists and antagonists on hormone release and cAMP production by purified islet (a and b) A cells and (c and d) B cells. The cells were incubated for 15 minutes under the same conditions as those outlined in the legend to Fig. 1, with the compounds to be tested present throughout the 15-minute incubation period. The results obtained with the agonists are shown in (a) and (c); epinephrine (E), norepinephrine (NE), methoxamine (ME), clonidine (CL), isoproterenol (IS) were tested at 10^{-7}*M*; control condition (C_1, no agonists added). Adrenergic antagonists (b and d) were tested at 10^{-6}*M* for their ability to block the effects of 10^{-7}*M* epinephrine; C_2, control condition containing only epinephrine; epinephrine plus prazosin (PZ), yohimbine (YO), phentolamine (PH), and propranolol (PR). Data are expressed as means ± SEM for four (A cells) and five (B cells) separate experiments. The significance of differences between agonist conditions and control C_1, and between antagonist conditions and control C_2, was calculated by the unpaired Student's t test and corrected by the Bonferroni method for multiple comparisons. $^*P < 0.01$.

islet preparations (*4, 5, 17, 18*). Our inability to detect β receptors on B cells and α receptors on A cells is, however, at variance with earlier studies in humans (*2*) and in isolated pancreatic tissue (*5, 18*). The absence of a second type of adrenergic recognition in purified A or B cells cannot be attributed to receptor damage for the following reasons: (i) all studies were carried out on cultured cells, which should have recovered from membrane damage; (ii) both α and β receptors were found to remain present on the purified cells, be it on different populations; (iii) the identified receptors were sensitive to catecholamine concentrations that were 10 to 100 times lower than those used in previous studies (*3–5, 17*); (iv) the purification process was previously shown to maintain high-affinity receptors on the membrane of purified cells (*19*). It seems likely, therefore, that the apparent discrepancy with previous reports is either related to species differences (*2*) or caused by misinterpretations in unpurified islet cell models.

Our results clarify the mechanisms involved in the adrenergic control of insulin and glucagon release and document the primordial role of the adrenergic tone on the secretory responsiveness of pancreatic A and B cells to nutrient stimuli. On the basis of these observations, a more specific approach can be undertaken to assess the participation of the adrenergic system in the pathogenesis and treatment of diabetes mellitus.

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Trans-Activator Gene of HTLV-II Induces IL-2 Receptor and IL-2 Cellular Gene Expression

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The human T-lymphotropic viruses types I and II (HTLV-I and -II) have been etiologically linked with certain T-cell leukemias and lymphomas that characteristically display membrane receptors for interleukin-2. The relation of these viruses to this growth factor receptor has remained unexplained. It is demonstrated here that introduction of the *trans*-activator (*tat*) gene of HTLV-II into the Jurkat T-lymphoid cell line results in the induction of both interleukin-2 receptor and interleukin-2 gene expression. The coexpression of these cellular genes may play a role in the altering T-cell growth following retroviral infection.

THE HUMAN T-LYMPHOTROPIC VIruses types I and II (HTLV-I and -II) are associated with certain T-cell leukemias and lymphomas (*1*). Both of these retroviruses can transform primary human $T4^+$ T cells in culture (*2*). Leukemic T-cell lines that are infected with HTLV-I or -II uniformly display large numbers of membrane receptors for interleukin-2 (IL-2) (*3*) and some, but not all, of these cell lines produce IL-2 (*4*). At present, the mechanism of transformation by these retroviruses remains unknown. However, neither HTLV-I nor HTLV-II contains a recognized oncogene nor do these viruses appear to activate cellular oncogenes by *cis* insertion of retroviral promoter (or enhancer) sequences, since the sites of proviral integration vary from tumor to tumor (*5*). An unusual feature of the HTLV-I and -II viruses is that they encode a gene whose protein product greatly stimulates the expression of other viral genes controlled by the long terminal repeat sequences (LTR's) (*6*). The elements of this autostimulatory control mechanism are the *trans*-activator proteins *tat*-I (42 kD) and *tat*-II (38 kD), which are encoded by a long open reading frame within the pX region (*7*) at the 3′ end of the viral genomes, and the *trans*-acting responsive sequences TAR-I and TAR-II, which are located within the viral LTR's (*8*). It has been suggested that the *tat* proteins may also be able to alter the expression of certain cellular genes involved in T-cell growth (*6, 9*). To test this possibility, we have introduced a functional *tat*-II gene into Jurkat T and Raji B cells and examined these cells for changes in the expression of cellular genes including IL-2 and the IL-2 receptor.

The *tat*-II gene from HTLV-II was isolated as a Bgl II–Bam HI fragment from pCATLORIIgpt (*6*) and inserted into the Bam HI site of the pZIPNEOSV(X) retroviral vector described by Cepko *et al.* (*10*). This plasmid has been described elsewhere (*11*). Plasmids containing the *neo* gene and *tat*-II in both the sense (pZIP-*tat*-II) and anti-sense (pZIP-anti-*tat*-II) orientation were identified and transfected into psi AM fibroblast cells (*12*) by calcium phosphate precipitation (*13*). Psi AM cells containing these plasmids were isolated by culture in medium containing the G418 antibiotic (400 μg/ml). The resultant amphotropic viruses produced by the *neo*-resistant psi AM cells were used to infect Jurkat T cells and Raji B cells (*11*). Infected Jurkat T- and Raji B-cell colonies were isolated by plating 10,000 cells per microliter well and culturing in G418 antibiotic (700 μg/ml).

Jurkat T and Raji B cells containing *tat*-II were first analyzed for the production of functional *tat*-II protein. Although derived

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