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Effects of Chronic Isoproterenol Administration on β_1-Adrenoceptors and Growth of Pancreas of Young and Adult Rats (42721)

CHARLOTTE A. SCHNEYER AND MICHAEL HUMPHREYS-BEHER

Department of Physiology and Biophysics and Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Abstract. [^{3}H]Dihydroalprenolol (DHA) binding of membranes of adult pancreas differed from that of pancreas of young rats, and the DHA binding in the presence of atenolol or butoxamine also was different in the two age groups. The adult pancreas had 93% β_2- and 7% β_1-adrenoceptors and did not exhibit an increased incorporation of [^{3}H]thymidine into deoxyribonucleic acid (DNA) following 2 days of DL-isoproterenol (ISO) administration; in contrast, pancreas of the 20-day-old rat had 71% β_2-adrenoceptors and 27% β_1-adrenoceptors and exhibited a 34-fold increase over that of adult, and a 6-fold increase over that of the control 20-day-old pancreas. Acinar cell differentiation was also accelerated by a 7-day regimen of ISO administration from 13 to 20 days of age. These growth responses to ISO appear to be β_1 mediated. The lack of β_1-adrenoceptors in the adult may account for the failure of the adult pancreas to exhibit a growth response to ISO.

The exocrine pancreas of adult rat does not exhibit a growth response to chronic administration of the β-adrenergic agonist, isoproterenol, whereas the parotid gland, which has structural and functional similarities to pancreas, shows a marked increase in size and number of acinar cells following such a drug regimen (1, 2). The glandular enlargement so induced is mediated by activation of β_1-adrenoceptors (3, 4), the predominant β-adrenergic subtype present in salivary glands (5). It appeared possible that the failure of adult exocrine pancreas to exhibit a growth response could be a consequence of its lack of β_1-adrenoceptors (6). Therefore, the first objective of the present work was to define the subtype of adrenergic receptor present in adult pancreas. Second, since it has been shown that the parotid and the submandibular glands of the early postnatal rats exhibit growth responses to chronic ISO administration that differ from those of glands of adult rats (7), it seemed possible that the pancreas of such rats would also exhibit responses to ISO that differed from those of the adult. This view seemed promising, especially since the β-adrenoceptors of the young and adult salivary glands also respond differently to chronic ISO administration (8). The characterization of the β_1–β_2-adrenergic receptor population of the pancreas of the young and adult rat was therefore undertaken, and the effect of chronic ISO administration on ratios of β_1–β_2 receptors in these organs was also assayed. Moreover, since accelerated differentiation is also induced in salivary glands of young rats by chronic administration of ISO (7), the effects of such a regimen on differentiation of acinar cells and their β-adrenoceptors were also examined.

Materials and Methods. Long–Evans rats ranging in age from 2 to 120 days were maintained on solid lab chow and water *ad lib.* Portions of pancreas and parotid glands were removed at varying ages, weighed rapidly on a torsion balance, and placed in 50 m*M* Tris–HCl buffer (pH 7.4) for subsequent receptor assays or for determination of thymidine incorporation into DNA or in Bouin's for subsequent histological examination. Some groups of rats were injected chronically with DL-isoproterenol (ISO) (50 mg/kg body wt) twice daily for periods of 2 days.

DNA synthesis *in vivo* in parotid glands and pancreas treated with ISO (50 mg/kg body wt) twice daily for 2 days was followed by monitoring the incorporation of 1 μCi [^{3}H]thymidine/100 g body wt into trichloroacetic acid-precipitable counts. The animals were killed 5 hr following the injection of [^{3}H]thymidine, and the tissues were removed and homogenized at 4°C. Samples (100 μl) were removed for precipitation with trichlo-

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roacetic acid on glass fiber filters, followed by scintillation counting, using Amersham premixed nonaqueous scintillation cocktail for ^3H incorporation. Part of the sample was removed for a protein assay. Duplicates of untreated animals were used to determine basal rates of synthesis.

Gland homogenates for both [^{3}H]quinuclidinylbenzilate (QNB) and [^{3}H]dihydroalprenolol (DHA) binding were prepared by centrifugation at 20,000g for 30 min (4°C). The pellet containing the membrane fraction was resuspended in 100 vol of 10 mM Tris–HCl buffer, pH 7.6, containing 4 mM $MgCl_2$ and 100 μM dithiothreitol. Membranes were resuspended by a combination of vortex vibration followed by Dounce homogenization. Protein concentrations were subsequently determined by a modification of the Lowry protein assay using bovine serum albumin as standard (9). Bindings of [^{3}H]QNB and [^{3}H]DHA were linearly dependent on membrane concentration within the dilution of both the pancreas and the parotid gland. Binding assays were performed in duplicate using 1.0 ml of diluted membrane and 1.0 nM [^{3}H]QNB or [^{3}H]DHA (10). The reaction mixture was incubated for 90 min at 37°C and terminated by the addition of 3 ml ice-cold 0.9% NaCl. Quantitation of binding was performed by precipitation of membranes from the above slurry onto glass fiber filters, washed three times with 5 vol of cold PSB, and counted for radioactivity by liquid scintillation. Nonspecific binding for QNB was determined by the inclusion of 1.0 μM atropine 10 min prior to the addition of labeled QNB. For DHA, nonspecific binding was determined using 10 μM propranolol in a 10-min preincubation step.

Histological sections were prepared from Bouin-fixed tissues. Sections 6 μm thick were stained with hematoxylin and eosin. Photomicrographs were made to show state of differentiation and acinar size. Student's t test was used for statistical analysis of data.

Results. The data in Table I show [^{3}H]DHA and [^{3}H]QNB binding of membranes of pancreas of adult and young rat. QNB binding of membranes from pancreas of adult and young rat was the same (about 32 pmole/mg membrane protein). DHA binding of membranes from pancreas of adult and young rat differed little from each other, except for the 14- and 20-day-old rats, where the difference was about 15%. When the reaction mixtures (containing adult pancreas) were preincubated with butoxamine, the β_2-adrenergic antagonist, DHA binding was virtually prevented and binding was only 7%; in the presence of atenolol, the β_1-adrenergic antagonist, DHA binding was almost complete (93%). These findings indicate that adult pancreas has mostly β_2-adrenoceptors.

The proportions of β_1- and β_2-adrenoceptors in the pancreas of the young rat differed

TABLE I. β_1 AND β_2 ADRENERGIC AND MUSCARINIC RECEPTORS IN PANCREAS OF ADULT (120 DAYS OF AGE) AND YOUNG (2–42 DAYS OF AGE) RATS

Age of rats (days)	No. of rats	β-Adrenoceptor density [^{3}H]DHA binding (fmole/mg membrane proein)			Muscarinic receptor density [^{3}H]QNB binding
		−Antag.	+Atenolol.	+Butoxamine	
2	7	58.1 ± 1.2	38.3 ± 0.3	18.0 ± 0.0	
7	6	60.7 ± 1.5	38.3 ± 0.7	17.3 ± 0.7	
10	12	58.6 ± 2.8	39.2 ± 1.4	16.8 ± 0.7	32.1 ± 0.9
14	9	52.1 ± 0.8*	39.3 ± 1.3	14.3 ± 0.5	32.4 ± 1.0
20	11	53.5 ± 0.9*	38.1 ± 1.2	14.0 ± 1.0	31.0 ± 1.2
28	12	61.0 ± 0.8	40.3 ± 0.6	16.4 ± 0.9	31.5 ± 1.0
35	11	59.0 ± 0.5	40.1 ± 0.7	18.0 ± 0.3	30.4 ± 0.8
42	4	59.1 ± 2.4	41.0 ± 1.3	18.0 ± 0.3	31.5 ± 0.3
120	18	63.0 ± 0.7	58.4 ± 1.2	4.7 ± 0.3	30.2 ± 0.4

Note. Values are means ± SE. Adult values for [^{3}H]DHA binding with atenolol or butoxamine present differ significantly ($P < 0.001$) from those of young rats in all cases: *Indicates adult values (no antagonists) differ from those of the 14- and 20-day-old rats ($P < 0.001$), but do not differ from values at all other ages. Values for QNB binding of pancreas of adult and young rats were the same.

TABLE II. EFFECTS OF CHRONIC ISOPROTERENOL ADMINISTRATION ON DENSITY OF ADRENOCEPTORS OF RAT PANCREAS

Age at sacrifice (days)	No. of rats	Treatment	β-Adrenoceptor density ([3H]DHA binding) (fmole/mg membrane protein)		
			−Antag.	+Atenolol	+Butoxamine
12	8	None	52 ± 1.9	39 ± 1.3	13 ± 0.8
	8	ISO	60 ± 1.7*	40 ± 0.8	20 ± 1.1*
25	10	None	60 ± 0.7	41 ± 0.9	15 ± 0.8
	10	ISO	53 ± 0.5*	39 ± 1.2	14 ± 0.1
33	8	None	59 ± 0.5	40 ± 0.7	18 ± 0.6
	7	ISO	57 ± 0.3	40 ± 0.6	17 ± 0.5

Note. Values are means ± SE. *Indicates value is significantly different ($P < 0.001$) from untreated. ISO is isoproterenol, given ip two times daily for 7 days, in 50 mg/kg body wt dose.

from those of the adult. As shown by data in Table I, at all postnatal ages from 2 to 42 days, DHA binding in the presence of butoxamine was 14–18 fmole/mg membrane protein and in the presence of atenolol, DHA binding was about 38–41 fmole/mg membrane protein. These data show that in the pancreas of young rat, approximately 30% of the β-adrenoceptor population is of the β_1 subtype and approximately 70% of the β_2 subtype. Chronic administration of isoproterenol to the young rats resulted, at most ages, in little change in total DHA binding; only at 12 and 25 days of age were the changes significant (15% increase at 12 days, and a 12% decrease in those 25 days of age at the time of sacrifice) (Table II). While DHA binding in the presence of atenolol or butoxamine was generally the same, with and without ISO, for the 12-day-old rats, there was a significant increase in β_1-adrenoceptors.

Chronic administration of ISO also elicited growth responses from pancreas of the young rats. The data in Table III show [3H]thymidine incorporation into DNA of pancreas of untreated and ISO-treated adult and young rats. The pancreas of adult untreated rat showed little [3H]thymidine incorporation into DNA; after 2 days of ISO administration, there was even less incorporation. However, the pancreas of the control 20-day-old rat showed a thymidine incorporation into DNA that was 6 times that of adult controls. The pancreas of 20-day-old rats given ISO for 2 days showed a striking increase in [3H]thymidine incorporation into DNA; it was 5.7 times that of the pancreas of 20-day-old controls, 35 times that of adult controls, and 75 times that of ISO-treated adult pancreas.

Parotid gland of the 120-day-old rat, after 2 days of ISO administration, showed a 4.4-fold increase in [3H]thymidine incorporation

TABLE III. EFFECTS OF ISOPROTERENOL ON [3H]THYMIDINE INCORPORATION INTO DNA OF PANCREAS AND PAROTID GLAND OF YOUNG AND ADULT RATS

Age of rats (days)	No. of rats	Treatment	[3H]Thymidine incorporation into DNA (cpm/mg protein)	
			Pancreas	Parotid
120	4	None	77 ± 3.1	306 ± 3.9
	4	ISO 2 days	36 ± 4.6*	1336 ± 38.0*
20	6	None	467 ± 71.8	316 ± 18.3
	6	ISO 2 days	2691 ± 164.8*	4293 ± 209.6*

Note. Values are means ± SE. DL-Isoproterenol administered ip two times daily for 2 days in a dose of 50 mg/kg body wt. *Indicates value is significantly ($P < 0.001$) different from control (no ISO treatment) for each age indicated.

into DNA and the parotid of the 20-day-old rat, treated for 2 days with ISO, showed a 13.6-fold increase when comparisons were made with glands of untreated rats (Table III). Moreover, while [^{3}H]thymidine incorporations into DNA of the parotid gland of untreated adult and untreated 20-day-old rat were the same, the thymidine incorporation into the parotid gland of the 20-day-old rat was 3.2 times that of adult parotid.

Differentiation of the pancreas of the young rat was, as with parotid, altered by chronic administration of ISO. The photomicrographs in Fig. 1 show that the acinar cells of the 20-day-old pancreas are larger than those of the 20-day-old controls (at least twofold increase, based on measurements with eyepiece micrometer) and differentiation of these cells from rounded cells, with nuclei in the center to pyramidal cells with nuclei basally located, was accelerated following 7 days of daily administration of ISO. Mitotic figures in acinar cells were numerous.

Discussion. Present data show that there is little age-associated change in density of β-adrenoceptors and none in muscarinic receptors of pancreas of young rats. Only in the 14- and 20-day age groups was DHA binding somewhat less than that of adults, a difference that may be related to functional changes seen at these ages. However, the proportions of β_1- and β_2-adrenoceptors in pancreas of the young rat differed sharply from the pattern seen in the adult pancreas. On an average, the proportions of β_1- and β_2-adrenoceptors at all ages examined from 2 to 42 days were respectively 27 and 71%; the adult pancreas on the other hand had 93% β_2- and 7% β_1-adrenoceptors. Thus, while total DHA bindings of pancreas of the young and adult rat were not very different, the ratios of β_1–β_2 receptors in the two age groups were.

Receptor density was not much altered by chronic treatment of the young rats with ISO, and only at 14 and 25 days of age were there modest changes (a small increase at 14 days, and a small decrease at 25 days). The increase may be associated with the accelerated maturation of the organ that was induced by the ISO; the rounded undifferentiated cells seen in the 20-day-old control were superseded by acinar cells of characteristic pyramidal shape with basally placed nuclei when ISO was given from Days 12 to 19. The decrease in DHA binding of ISO-treated pancreas seen at 25 days corresponds to the down-regulation of β-adrenoceptors seen in adult parotid gland after 4–9 days of ISO treatment (12). However, while the differences in response at 14 and 25 days may be a reflection of the differing states of maturity of the pancreas, the lack of change in the 33-day-old rat casts some doubt on this interpretation. It remains possible that the small changes at 14 and 25 days are within experimental error.

The growth response of the pancreas of the young rat to chronic administration of ISO differs sharply from that of adult pancreas. In the young rat, the incorporation of [^{3}H]thymidine into pancreatic DNA was high in the untreated rat when comparison was made with the adult, but when ISO was given for 2 days, it was 35 times that of adult control and nearly 6 times that of the 20-day-old control. Thus, the pancreas of the young rat shows a growth response that mimics that seen in parotid and submandibular glands following treatment with ISO, whereas the pancreas of the adult does not. The parotid and submandibular glands have about 95% β_1- and 5% β_2-adrenoceptors (3), and the growth response of the salivary glands to ISO is attributed to activation of the β_1-adrenoceptors (3, 4). It now appears that the failure of the pancreas of adult rat to respond to ISO may be attributed to the virtual lack of β_1-adrenoceptors. On the other hand, the increased DNA synthesis and accelerated development of pancreas in the young rat in response to ISO can be attributed to the significant proportion of β_1-adrenoceptors present during early development. ISO treatment of the young rat also results in an accelerated development of parotid and submandibular glands that is attributed to activation of β_1-adrenoceptors (7, 11). A change in number of β-adrenoceptors also accompanies the ISO-induced acceleration of acinar cell development, and on the basis of present data, such changes may accompany the pancreatic developmental acceleration (8). Experiments are in progress to establish this point more definitely.

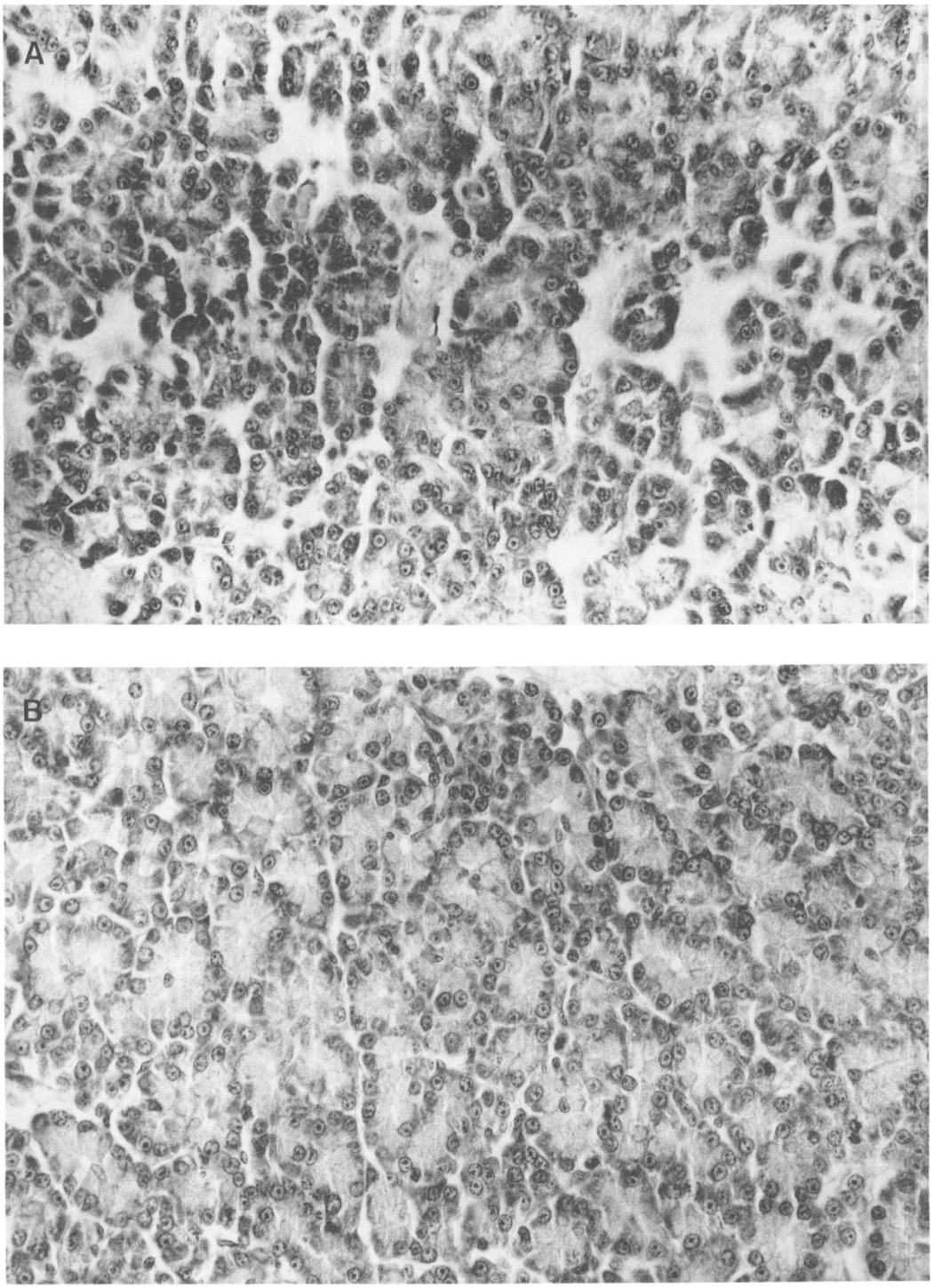

FIG. 1. (A) Photomicrograph of pancreas of 20-day-old rat, untreated. Acinar cells are not fully differentiated (mag. ×484). (B) Photomicrograph of pancreas of 20-day-old rat, following 7 days of twice daily injection of 50 mg/kg body wt doses of DL-isoproterenol. Acinar cells show significant increase in size and differentiation from those of untreated glands (mag. ×484).

Finally, it appears probable that the growth responses of the pancreas of young rats are mediated by β_1-adrenoceptors of the exocrine rather than of the endocrine portion of this organ. First, the acceleration of development of presumptive acinar cells into mature ones, accompanied by a more homogeneous appearance of the parenchyma, suggests that the ISO affects the exocrine pancreas. Second, there are only 1–2% islet (13) cells in the adult pancreas, and this percentage is too small to account for the marked differences in DNA synthesis.

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