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HISTAMINERGIC REGULATION OF PANCREATIC ß-CELL REPLICATION AND INSULIN SECRETION

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Evidence suggests that histamine is required for the diabetogenic agent streptozotocin to exert its toxicity on islet ß-cells. The effects of histamine and L-histidine on the replication and long-term insulin secretion by pancreatic ß-cells were investigated. L-histidine dose-dependently increased insulin secretion and suppressed DNA synthesis without affecting the islet insulin content. Histamine suppressed ß-cell replication but failed to affect the islet content or secretion of insulin. Depletion of islet histamine contents by the specific and irreversible inhibitor of L-histidine decarboxylase, α-fluoromethyl-[S] histidine increased islet insulin content but failed to influence the rate of insulin secretion. The present results suggest that exogenously added L-histidine, but not histamine, stimulates insulin secretion whereas both substances suppress ß-cell growth. Endogenously formed histamine may have different roles in ß-cell function than exogenously delivered histamine, the latter likely acting through specific cell surface receptors.

The capacity of the insulin-producing pancreatic ß-cell to alter its proliferative activity in response to an increased functional demand may be of decisive importance for maintenance of normoglycemia. Thus, an increased ß-cell mass may represent a long-term adjustment to meet an elevated blood glucose level, contrasting to the rapid disposal of the sugar brought about by an increased rate of insulin synthesis and release from individual ß-cells (1,2). In contrast to the low proliferative compartment of adult ß-cells, modulation of the proliferative activity can more easily be accomplished in undifferentiated fetal ß-cells (1). Stimulation of fetal ß-cell proliferation can be induced in tissue culture by glucose, amino acids and polypeptide growth factors (3,4). On the other hand, ß-cell specific cytotoxins, such as the diabetogenic agent streptozotocin cause the destruction of the ß-cell population (5) and decreases ß-cell growth (6). Previous reports indicate that streptozotocin gives rise to an early and large increase in islet histamine content (7), and that the toxicity of the drug required vasoactive amine release (8). It is thus possible that endogenous histamine synthesis is an important event mediating the cytotoxicity of streptozotocin. It was suggested that histamine may be involved in a vicious circle in the ß-cell (7) in which streptozotocin activates xanthine oxidase (9), generating oxygen radicals which in turn stimulate formation of histamine (10) that subsequently activates xanthine oxidase (11). In the present study, long-term effects of exogenous L-histidine and histamine on pancreatic ß-cell replication and insulin secretion were evaluated and compared to effects of depletion of endogenous histamine.

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MATERIALS AND METHODS

MATERIALS: α-Fluoromethyl-[S]-histidine (FMH) was graciously donated by Dr. J. Kollonitsch, Merck Sharp & Dohme, Rahway, NJ, U.S.A. L-histidine, histamine and Hepes were obtained from Sigma Chemicals, St. Louis, MO, U.S.A. Collagenase type CLS (EC 3.4.24.3) was obtained from Boehringer-Mannheim, Mannheim, Germany. Culture medium RPMI 1640, fetal calf serum, L-glutamine, bensylpenicillin and streptomycin were from Flow Laboratories, Irvine, U.K. Antibovine insulin serum was supplied by Miles-Yeda, Rehovot, Israel. Crystalline mouse insulin and ^{125}I-insulin were from Novo, Copenhagen, Denmark. [*Methyl*-^{3}H]thymidine (5 Ci/mmol) was procured from Amersham International, Bucks., U.K. Unisolve was from New England Nuclear, Boston, MA, U.S.A. and Soluene was provided by Packard Instruments, Downers Grove, IL, U.S.A.

PREPARATION AND CULTURE OF FETAL ISLETS: Pregnant Sprague-Dawley rats belonging to a local stock were killed by cervical dislocation on day 21 of gestation and the fetuses rapidly removed. Fetal rat islets were prepared from pancreatic glands as previously described (12). Briefly, the pancreata were finely chopped and digested for a short time with collagenase. The carefully washed digest was plated in culture dishes allowing cell attachment (Nunc, Roskilde, Denmark) and cultured for 5 days at 37 °C in a humidified atmosphere of 5 % CO_2 in ambient air in medium RPMI 1640 containing 11.1 mM glucose, 10 % fetal calf serum, 2 mM L-glutamine, 100 U/ml bensylpenicillin and 0.1 mg/ml streptomycin. At the end of the culture period, groups of islets were transferred to fresh media containing 1 % fetal calf serum and cultured free-floating over-night, a procedure that minimizes fibroblast proliferation. Spherical islets, free of connective tissue, were then selected under a stereo microscope and used for the different analysies listed below.

DNA SYNTHESIS AND DNA CONTENT: Islets in groups of 50 were cultured for 1-3 days as described above. During the last 5 h, 1 μCi/ml of [*methyl*-^{3}H]thymidine was present in culture media. At the end of the labeling period the islets were washed in PBS, ultrasonically homogenized and acid-insoluble material pelleted by centrifugation in ice-cold 10 % trichloroacetic acid. The precipitate was washed twice in trichloroacetic acid and dissolved in 50 μl of Soluene. The radioactivity incorporated was determined by scintillation counting after addition of 1 ml of Unisolve. Given the long cell cycle of the ß-cell (13), and the fact that unsynchronized cells were studied, it was considered necessary to expose the islets for 1-3 days to the different test substances, a procedure that allows DNA synthesis initiated prior to addition of test substances to be terminated before [^{3}H]thymidine addition. Duplicate samples of the homogenate were analyzed fluorometrically for DNA (14,15).

ISLET INSULIN CONTENT AND INSULIN SECRETION: The islet insulin content in homogenates extracted overnight at 4 °C in 0.18 M HCl/70 % ethanol (16) and insulin accumulation in culture media during the last 24 h of culture were determined radioimmunologically (17).

RESULTS

As shown in Figure 1, after 3 days of exposure to both L-histidine and histamine decreased ß-cell DNA synthesis, as assessed by measurements of [^{3}H]thymidine incorporation into DNA, at concentrations of 10 mM. Lower concentrations produced minor, insignificant effects.The apparent increase in DNA synthesis by 1 mM of L-histidine was not statistically significant and likely reflects a random difference. On the other hand, long-term insulin secretion was dose-dependently enhanced by L-histidine, significant effects being recorded already at 0.1 mM (Fig. 2). By contrast, histamine (0.1-10 mM) had no effect whatsoever on long-term insulin secretion. Depletion of endogenous histamine pools, by treatment with the specific and irreversible L-histidine decarboxylase inhibitor FMH (1 mM), resulted in a significant increase in islet insulin content whereas the secretion of the hormone remained unaltered by this treatment (Fig. 3). Neither L-histidine nor histamine, at any concentration examined, seemed to exert any cytotoxic action in this system, since the islet insulin content remained unaltered (not shown), averaging 1015 ± 112 ng/50 islets (n=4).

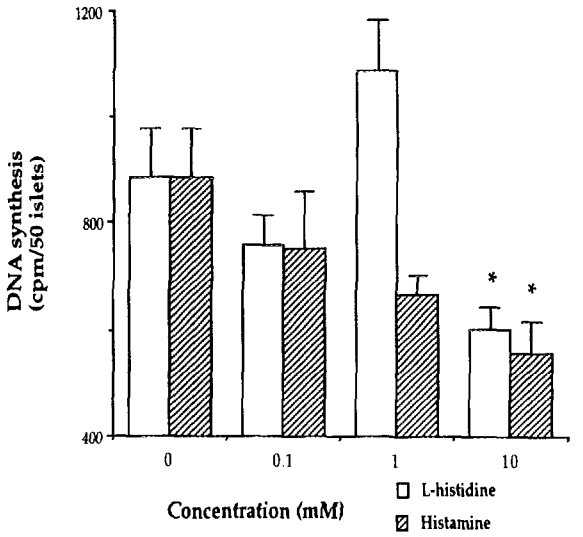

Figure 1. Histamine and L-histidine decreases ß-cell proliferation.
Islets were cultured for 3 days as indicated in the Figure. During the final 5 h of culture, 1 µCi/ml [^{3}H]thymidine was present in culture media and DNA synthesis rates were determined by measuring the radioactivity incorporated into trichloroacetic-acid-precipitable material. Bars represent means ± S.E.M. for 4 observations. * denotes P<0.05 for a chance difference *vs.* control islets using Student's unpaired *t*-test.

DISCUSSION

Long-term alterations in ß-cell mass constitute an important means to accommodate an increased demand for insulin. Since previous studies have established a defective insulin secretory response to glucose (18) as well

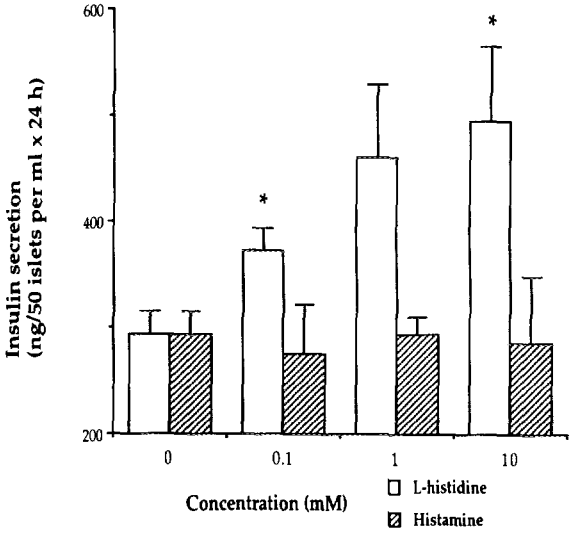

Figure 2. L-histidine, but not histamine, dose-dependently promotes insulin release.
Islets were cultured for 3 days as indicated in the Figure.. The basal concentration of L-histidine in culture medium RPMI 1640 is ≈ 97 µM. The secretion of insulin into the culture medium during the final 24 h of culture was determined radioimmunologically. Bars represent means ± S.E.M. for 4 observations. * denotes P<0.05 for a chance difference *vs.* control islets using Student's unpaired *t*-test.

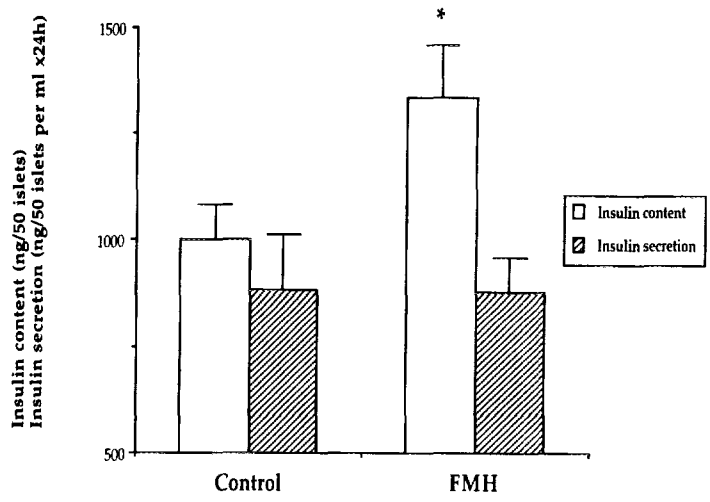

Figure 3. Histamine depletion increases islet insulin content.
Islets were cultured for 3 days as indicated in the Figure with or without 1 mM FMH. The islet insulin content extracted in acid ethanol and the secretion of insulin into the culture medium during the 24 h of culture were determined radioimmunologically. Bars represent means ± S.E.M. for 4 observations. * denotes $P<0.05$ for a chance difference *vs.* control islets using Student's unpaired *t*-test.

as a decreased ß-cell volume (1,2) in diabetic patients, further elucidation of factors governing insulin production and proliferation of the healthy ß-cell is clearly warranted. Neither in human or animal models for typ-1 diabetes, nor in type-2 diabetes, is ß-cell regeneration a noteworthy feature (1,2). Thus, if ß-cells could be induced to replicate at a higher rate, this may prove beneficial in maintaining normoglycaemia, since the ß-cell mass is a major determinant of the total amount of insulin that can be secreted by the pancreas. Importantly, when ß-cell population is expanded *in vivo* by "cellophane wrapping" of the pancreas, this not only induces islet hyperplasia resembling nesidioblastosis, but also ameliorates experimental diabetes in hamsters (19).

In the present study, long-term effects of exogenous L-histidine and histamine on pancreatic ß-cell replication and insulin secretion were evaluated and compared to effects of depletion of endogenous histamine. By comparison to other biogenic amines, *e.g.* polyamines (3), not much is known about the role of histamine in the ß-cell. However, reports have suggested that exogenous histamine (0.1-1 mM) does not influence secretion of insulin from isolated rat islets (20,21), findings reproduced in the present study investigating long-term effects. Given the recent development of FMH, a specific inhibitor of the pace-setting enzyme L-histidine decarboxylase (22,23), pharmacological depletion of intracellular histamine has become amenable and enables us to conclusively address the issue of functional significance of histamine in islets. FMH potently and irreversibly inhibits L-histidine decarboxylase, exhibiting a K_i of 8.3 µM (23). Evidence is accumulating that histamine may be involved in the regulation of cell proliferation and tumor development (24,25). There is evidence suggesting that exogenous histamine, probably by signaling through cell surface H_1-receptors, can decrease tumor growth (25-27). Conceivably, this mechanism may explain why histamine inhibits DNA synthesis rates in the presently studied ß-cells.

In comparison to other amino acids, information concerning the interaction of L-histidine with islet ß-cells is scarce. Nonetheless, previous investigators have shown that infusion of the amino acid into healthy subjects did not influence serum insulin levels (28). Among *in vitro* studies, a lack of insulinotropic effect of L-histidine was reported in some cases (20,29,30), whereas at intermediate glucose concentrations the amino acid caused a rapid and sustained increase in insulin output (31-33). The mechanism by which L-histidine exerts its insulinotropic action, however, remains largely elusive since work by Sener *et al.* (33) showed that it seemed unrelated to changes in islet respiration, histamine generation, or changes in transglutaminase activity. Although an increased Ca^{2+} entry was detected after L-histidine exposure it is conceivable that exogenous L-histidine evokes insulin secretion in part owing to its weakly basic imidazolium function.

Analysis of the functional consequences of exhaustion of endogenous histamine pools by FMH revealed a totally different scenario than the actions of exogenously added amine. Thus, the finding that FMH-treated islets displayed elevated insulin contents, but not increased insulin secretory rates, would seem to suggest a selective inhibitory role for endogenous histamine in some step of insulin production. Although early investigators showed that islets contain biogenic amines (34), probably located in the secretory granules, their functional role has remained elusive. It is furthermore likely that cell surface H_1- or H_2-receptors convey the effects of exogenous histamine, the function of which may thus be totally unrelated to that of endogenous histamine.

In their entirety, the present results suggest that exogenously added L-histidine, but not histamine, stimulates insulin secretion whereas both substances suppressed ß-cell growth. Endogenously formed histamine may have different roles in ß-cell function (possibly an inhibitory role in insulin production) than exogenously delivered histamine, the latter likely acting through specific cell surface receptors.

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