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Monoamines in the Pancreatic Islets of the Mouse*

5-Hydroxytryptamine as an Intracellular Modifier of Insulin Secretion, and the Hypoglycaemic Action of Monoamine Oxidase Inhibitors

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Summary. The functional significance of 5-hydroxytryptamine (5-HT) storage in the pancreatic B cells for insulin secreting mechanisms was studied in normal mice *in vivo*. Pretreatment of the animals with *L*-5-hydroxytryptophan (*L*-5-HTP) markedly decreased the insulin releasing capacity after sulphonylurea stimulation. This inhibition of insulin release could be abolished by previous administration of an inhibitor of aromatic amino acid decarboxylation. On the other hand, pretreatment with the monoamine oxidase inhibitor nialamide alone, decreased sulphonylurea-induced insulin release. The combined treatment with nialamide and *L*-5-HTP did not further decrease the insulin response. Insulin release induced by *L*-isopropylnoradrenaline (*L*-IPNA) was also found to diminish after previous administration of *L*-5-HTP or nialamide; but, unlike the insulin response to sulphonylurea, insulin release induced by IPNA could be totally suppressed by the combined treatment of nialamide or pargyline and *L*-5-HTP. Insulin release induced by glucose was not significantly influenced with any of the above treatments. Basal levels of plasma insulin were not affected by *L*-5-HTP injection, and were not consistently diminished by the combined treatment with monoamine oxidase inhibitor and *L*-5-HTP. The combined treatment with monoamine oxidase inhibitors and *L*-5-HTP was found to elicit a profound hypoglycaemia in both normal and alloxan-diabetic mice. The hypoglycaemic condition was accompanied by exhaustion of liver and muscle glycogen. The hypoglycaemia could be abolished by previous treatment with an inhibitor of aromatic amino acid decarboxylation. Combined treatment with pargyline and 5-HT brought about a marked hyperglycaemia. It is concluded that: 1. intracellular levels of 5-HT in the pancreatic B cells possess the ability to modify insulin secreting mechanisms; and 2. the hypoglycaemic action of monoamine oxidase inhibitors is brought about by raised intracellular levels of 5-HT, which is accompanied by a markedly increased glucose utilization by the tissues.

Monoamines dans les îlots pancréatiques chez la souris

Résumé. Chez la souris normale a été étudiée *in vivo* la signification fonctionnelle du stockage de 5-hydroxytryptamine (5-HT) dans les cellules β du pancréas pour les mécanismes de la sécrétion d'insuline. Un traitement préalable des animaux avec le *L*-5-hydroxytryptophane (*L*-5-HTP) a nettement réduit la capacité de sécrétion d'insuline après stimulation par sulfonylurée. Cette inhibition de la sécrétion d'insuline pouvait être évitée par l'administration préalable d'un inhibiteur de décarboxylation d'acide aminé aromatique. D'un autre côté, le traitement préalable avec la nialamide, inhibiteur de la monoamine oxydase, réduisait la sécrétion d'insuline provoquée par sulfonylurée. Le traitement combiné avec la nialamide et le *L*-5-HTP n'a pas réduit davantage la réponse de l'insuline. Il a été trouvé que la sécrétion d'insuline provoquée par la *L*-isopropylnoradrénaline (*L*-IPNA) se réduisait également après l'administration préalable de *L*-5-HTP ou de nialamide, mais, contrairement à la réponse de l'insuline après sulfonylurée, la sécrétion d'insuline provoquée par l'IPNA pouvait être totalement supprimée par le traitement combiné avec la nialamide ou la pargyline et le *L*-5-HTP. La sécrétion d'insuline provoquée par le glucose n'était influencée de façon significative par aucun des traitements ci-dessus. Le taux basal d'insuline du plasma n'était pas affecté par l'injection de *L*-5-HTP et n'était pas réduit de façon certaine par le traitement combiné avec l'inhibiteur de la monamine oxydase et le *L*-5-HTP. Il a été trouvé que le traitement combiné avec l'inhibiteur de la monoamine oxydase et le *L*-5-HTP provoquait une hypoglycémie profonde à la fois chez la souris normale et chez la souris diabétique par l'alloxane. L'hypoglycémie était accompagnée d'un épuisement du contenu du glycogène du foie et des muscles. Il était possible d'éviter l'hypoglycémie par un traitement préalable avec un inhibiteur de décarboxylation d'acide aminé aromatique. Un traitement combiné avec la pargyline et la 5-HT a provoqué une nette hyperglycémie. — En conclusion: 1. Le taux intracellulaire de la 5-HT dans les cellules β du pancréas a la capacité de modifier les mécanismes de la sécrétion d'insuline. 2. L'action hypoglycémique des inhibiteurs de la monoamine oxydase est provoquée par l'accroissement du taux intracellulaire de 5-HT qui s'accompagne d'une nette augmentation de l'utilisation du glucose par les tissus.

Monoamine in den Pankreasinseln der Maus

Zusammenfassung. Es wurde bei normalen Mäusen *in vivo* die funktionelle Bedeutung der Speicherung von 5-Hydroxytryptamin (5-HT) in den B-Zellen des Pankreas für die Mechanismen der Insulinsekretion untersucht. Eine Vorbehandlung der Tiere mit *L*-5 Hydroxytryptophan (*L*-5-HTP) verminderte deutlich die Insulinsekretion nach Stimulation mit Sulfonylharnstoff. Diese Hemmung der Insulinsekretion konnte durch vorherige Behandlung mit einem Hemmer der aromatischen Aminosäurendekarboxylase verhindert werden. Andererseits wurde die durch Sulfonylharnstoff bewirkte Insulinsekretion nach alleiniger Vorbehandlung mit dem Monoaminooxidasehemmer Nialamid vermindert. Die kombinierte Behandlung mit Nialamid und *L*-5-HTP hat die Insulinantwort nicht weiter gemindert. Die durch *L*-Isopropylnoradrenalin (*L*-IPNA) bewirkte Insulinausschüttung wurde ebenfalls nach einer vorherigen Behandlung mit *L*-5-HTP oder Nialamid reduziert. Aber im Gegensatz zu der Insulinantwort nach Sulfonylharnstoff konnte die durch IPNA induzierte Insulinausschüttung völlig durch die kombinierte Behandlung mit Nialamid oder Pargylin plus *L*-5-HTP unterdrückt werden. Die durch Glucose herbeigeführte Insulinausschüttung wurde nicht wesent-

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lich durch eine der oben erwähnten Behandlungen verändert. Die basale Plasmainsulinkonzentration wurde durch die *L*-5-HTP-Injektion nicht beeinflußt und war auch nicht wesentlich durch die kombinierte Behandlung mit dem Monoaminooxidasehemmer und *L*-5-HTP vermindert worden. — Die kombinierte Behandlung mit Monoaminooxidase-Inhibitoren und *L*-5-HTP erzeugte eine tiefe Hypoglykämie in normalen und alloxandiabetischen Mäusen. Der hypoglykämische Zustand wurde von einem Verschwinden des Leber- und Muskelglykogens begleitet. Die Hypoglykämie konnte durch eine Vorbehandlung mit einem Inhibitor der aromatischen Aminosäuredekarboxilation verhindert werden. Die kombinierte Behandlung mit Pargylin und 5-HT führte zu einer starken Hyperglykämie. — Daraus wurde geschlossen, 1. daß die intrazelluläre Konzentration von 5-HT in den B-Zellen des Pankreas die Fähigkeit besitzt, den Mechanismus der Insulinsekretion zu beeinflussen, 2. daß die hypoglykämische Wirkung der Monoaminooxidase-Inhibitoren durch eine erhöhte intrazelluläre 5-HT-Konzentration erzeugt wird, welche von einer stark erhöhten Glucoseutilisation der Gewebe begleitet wird.

Key words: 5-hydroxytryptamine, 5-hydroxytryptophan, monoamine oxidase inhibition, decarboxylase inhibition, glucose, glibenclamide, isopropylnoradrenaline, alloxan diabetes, mouse, blood glucose, immunoreactive insulin, tissue glycogen, hypoglycaemia.

Autoradiographic investigations have revealed a specific uptake of 5-hydroxytryptophan (5-HTP) in the pancreatic islets of the mouse [10, 24]. In a previous paper [6] evidence was presented, by means of electron-microscopic autoradiography, that in the B cells of mouse islets, labelled 5-hydroxytryptamine (5-HT), formed from the administered precursor ^{3}H-5-HTP, was confined to the specific granules assumed to store insulin and (or) insulin precursors. This finding raised the question whether 5-HT could play a biological role in the regulation of insulin secretion *in vivo*. Previous work on this problem has mainly been done *in vitro* on preparations from different mammalian species, and has so far given conflicting results [7, 8, 25, 27].

The aim of the present investigation was to study basal and stimulated insulin secretion *in vivo* after a preceding intravenous injection of the amine precursor *L*-5-HTP at a time when the 5-HT stores associated with insulin secretion granules were assumed to be maximally loaded [6]. In addition, the effect of monoamine oxidase inhibitors on insulin secretion and blood glucose level was studied.

Materials and Methods

Animals: Female mice of the NMRI strain (Lab. Animal Breeding, Laven, Denmark) weighing 20—25 g were used (total number: 500). The animals were kept on a standard pellet diet (Ferrosan Ltd., Malmö, Sweden) and tap water *ad libitum*. They had free access to food and drinking water before and throughout all experiments.

Drugs: *L*-5-hydroxytryptophan and 5-hydroxytryptamine creatinine sulphate were obtained from Fluka AG, Buchs, Switzerland. *L*-isopropylnoradrenaline (IPNA) as the bitartrate was a generous gift from Hässle AB, Göteborg, Sweden, nialamide from Chas Pfizer & Co., USA, glibenclamide from Boehringer Mannheim GmbH, Germany, pargylinehydrochloride from Abbot Lab., USA and Ro 4-4602 as the hydrochloride from Hoffmann-La Roche Ltd., Basel, Switzerland. All other drugs and chemicals were obtained from British Drug Houses Ltd., Poole, England.

Experimental: *L*-5-HTP (0.03, 0.07 or 0.26 mmol/kg body weight), *L*-IPNA (1.37 μmol/kg), glibenclamide (0.5 μmol/kg) and glucose (8.33 mmol/kg) were always injected into a tail vein. Nialamide (0.27 mmol/kg), pargyline (0.27 mmol/kg) and Ro 4-4602 (0.85 mmol/kg) were given intraperitoneally. Alloxan diabetes was induced in non-fasted animals by intravenous injection of 0.44 mmol/kg of alloxan monohydrate as previously described [22]. Alloxan-diabetic animals were used within 2 months after alloxan injection, and had an initial blood glucose level of at least 300 mg/100 ml. All drugs were administered in 0.9% NaCl in a volume of 0.2 ml/20 g mouse. Blood sampling was performed by orbital puncture using commercial constriction pipettes as described previously [21]. No anaesthesia was used during the experiments, and the mice were allowed to move around freely in their cages between samplings.

Blood glucose was determined enzymatically [17]. The concentrations of insulin in plasma were determined by the method of Heding [12] using ^{125}I-labelled pig insulin and guinea pig anti-pig-insulin. The immunoassay kit was generously provided by Dr. L. Heding, Novo Research Institute, Copenhagen, Denmark. Tissue glycogen concentrations were determined according to Rerup and Lundquist [23]. The enzyme preparation used for the direct measurement of glycogen obtained by KOH-ethanol fractionation was "Fermcozyme CB-B" (Hughes & Hughes Ltd., Brentwood, England). Student's *t*-test was employed for tests of significance.

Results

Effect of an intravenous injection of L-5-HTP on basal and stimulated insulin release

The first series of experiments (Fig. 1) was designed to elucidate the influence of exogenously administered *L*-5-HTP on plasma insulin and blood glucose levels after injection of saline (0.9% NaCl), glucose, glibenclamide and *L*-IPNA, respectively. *L*-5-HTP (0.26 mmol/kg body weight) was given one hour prior to the injection of saline or insulin-releasing agent.

Previous investigations in this laboratory have shown that maximum concentration of insulin in mouse plasma following a rapid intravenous injection of glucose, the highly potent sulphonylurea compound glibenclamide, and the beta-adrenergic stimulator *L*-IPNA, is achieved after 2—2.5 min (glucose), 1.5—2.5 min (glibenclamide) and 5—6 min (*L*-IPNA). Accordingly, eight groups of mice were pretreated with either saline or *L*-5-HTP, and then injected with saline or one of the insulin-releasing agents. Plasma insulin and blood glucose levels were measured after 2 min (glucose and glibenclamide) and 5.5 min (*L*-IPNA). Fig. 1 shows that *L*-5-HTP had no effect on basal insulin or blood glucose levels. No effect of

L-5-HTP on glucose-mediated insulin release was recorded; but a significant decrease in the concentration of plasma insulin was noted in animals pretreated with *L*-5-HTP, following glibenclamide ($p < 0.001$) and *L*-IPNA ($p < 0.05$). No differences were recorded in the concentration of blood glucose between controls pretreated with saline, and animals pretreated with *L*-5-HTP. It must be emphasized that the *initial* level of blood glucose was determined in each animal always immediately *before* injection of the insulin-releasing agent. The *final* blood glucose sample was taken 2—5.5 min later (together with the plasma insulin sample). There was never any change in blood glucose level during this short time interval irrespective of injected agent (except glucose), and therefore only the *final* blood glucose values are illustrated in the figures from this type of experiment.

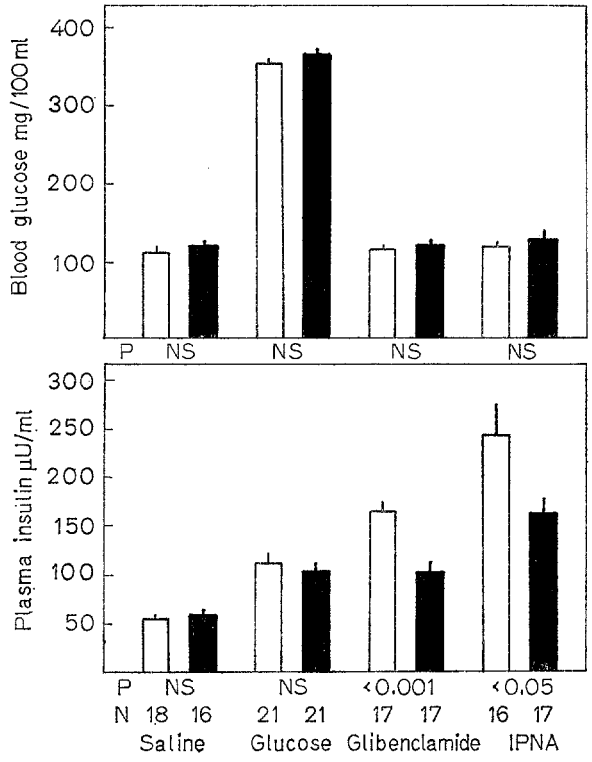

Fig. 1. Blood glucose levels and immunoreactive plasma insulin levels after intravenous injection of saline, glucose (8.33 mmol/kg), glibenclamide (0.5 µmol/kg), and *L*-IPNA (1.37 µmol/kg), respectively. Pretreatment with saline (white columns) or *L*-5-HTP, 0.26 mmol/kg (black columns), was given one hour prior to the experiment. Vertical bars indicate standard error of the mean. *P* = probability level of random difference. NS = not significant. N = number of animals in each group

Effect of the monoamine oxidase inhibitor nialamide on basal and stimulated insulin release

In a second series of experiments (Fig. 2) the effect of nialamide on basal, glucose-mediated, and glibenclamide-mediated insulin secretion was studied. Pretreatment with nialamide was given by two injections 18 h and 2 h prior to experiment. Groups of animals given *L*-5-HTP in addition to nialamide were injected with this agent one hour prior to experiment as described above. Fig. 2 illustrates that two injections of nialamide brought about a slight but significant reduction of the basal level of plasma insulin ($p < 0.025$), and a slight but highly significant decrease of the basal level of blood glucose ($p < 0.001$). The combined treatment with nialamide and *L*-5-HTP (0.26 mmol/kg) induced a profound hypoglycaemia (mean blood glucose level 14 ± 4 mg/100 ml), but had no significant effect on the level of plasma insulin. Several mice in this group showed signs of convulsions. These were partly relieved by glucose administration, but central disturbances of possibly non-hypoglycaemic origin cannot be excluded [26]. No effect on glucose-mediated insulin release was noted either with nialamide alone or with nialamide combined with a subsequent injection of *L*-5-HTP. The significant decrease in the level of blood glucose after the latter treatment

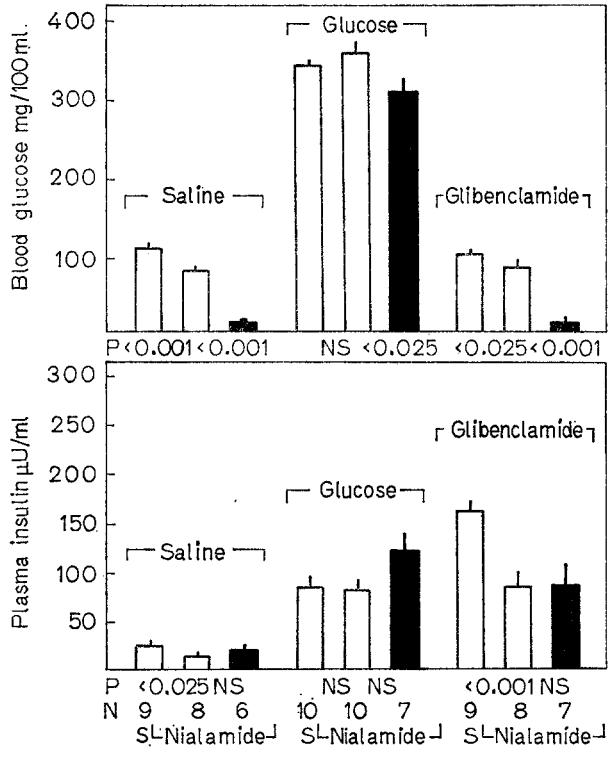

Fig. 2. Blood glucose levels and immunoreactive plasma insulin levels after intravenous injection of saline, glucose (8.33 mmol/kg), and glibenclamide (0.5 µmol/kg), respectively. Pretreatment with saline (S) or nialamide (0.27 mmol/kg) was given by two injections of the above dose at −18, and −2 h before the experiment. *L*-5-HTP, 0.26 mmol/kg (black columns) or saline (white columns) was given one hour prior to the experiment. Vertical bars indicate standard error of the mean. *P* = probability level of random difference. NS = not significant. N = number of animals in each group

compared with the other two glucose-injected groups (Fig. 2) depended on the extremely low level of the *initial* blood glucose elicited by nialamide + *L*-5-HTP treatment. The mean *rise* in the level of blood glucose after glucose injection was not significantly different among the three groups. The glibenclamide-injected animals showed a significantly ($p<0.001$) reduced insulin response after nialamide pretreatment. No further decrease of glibenclamide-stimulated insulin secretion was noted after the combined treatment with nialamide and *L*-5-HTP, in spite of the remarkable hypoglycaemia, which was already present at the time of glibenclamide injection.

Effect of various doses of nialamide and L-5-HTP on L-IPNA-induced insulin release

A third series of experiments was designed for a study of *L*-IPNA-provoked insulin secretion after: 1) two injections of nialamide; 2) two injections of nialamide and an additional dose of *L*-5-HTP (0.26 mmol/kg); and 3) one injection of nialamide and a small additional dose of *L*-5-HTP (0.03 mmol/kg). Fig. 3 illustrates the results. Groups of animals given the treatment of one acute injection of nialamide (−2 h) and the small dose of *L*-5-HTP (0.03 mmol/kg) are marked by an asterisk in Fig. 3. Otherwise, nialamide and *L*-5-HTP treatments were performed as described above for the second series of experiments. From Fig. 3 it appears that one injection of nialamide combined with the small dose of *L*-5-HTP significantly decreased the basal levels of blood glucose and plasma insulin. After pretreatment with two injections of nialamide alone a diminished response of plasma insulin following *L*-IPNA was recorded. The level of blood glucose was slightly but significantly decreased ($p<0.001$) as previously noted (Fig. 2). In contrast to glibenclamide-induced insulin release (Fig. 2), the response of the plasma insulin following *L*-IPNA was further decreased ($p<0.001$) after the combined treatment of two nialamide injections and *L*-5-HTP (0.26 mmol/kg). One injection of nialamide plus the small dose of *L*-5-HTP was found to decrease significantly ($p<0.001$) the insulin response after *L*-IPNA compared with saline-pretreated controls. It is worth noting that the concentration of blood glucose after one injection of nialamide plus the small dose of *L*-5-HTP is significantly ($p<0.02$) higher than after two injections of nialamide plus the large dose of *L*-5-HTP, whereas the level of plasma insulin is not.

Action of pargyline, a non-hydrazine monoamine oxidase inhibitor; and the effect of an inhibitor of aromatic amino acid decarboxylase

Because of certain "side effects" of hydrazine compounds on carbohydrate metabolism [28, 19], the effect of the non-hydrazine monoamine oxidase inhibitor, pargyline, was studied on *L*-IPNA-induced insulin release. One injection of pargyline (−2 h) or one injection of pargyline plus the small dose of *L*-5-HTP (0.03 mmol/kg) did not influence *L*-IPNA-mediated insulin release (Fig. 4a). However, after three injections of pargyline (−25, −18 and −2 h) combined with 0.26 mmol/kg of *L*-5-HTP (Fig. 4a; marked by an asterisk), the insulin response to *L*-IPNA was almost abolished (not significantly different from basal insulin levels) but significantly different from *L*-IPNA-injected controls ($p<0.01$). A profound hypoglycaemia, as was previously noted with nialamide plus *L*-5-HTP, was also recorded.

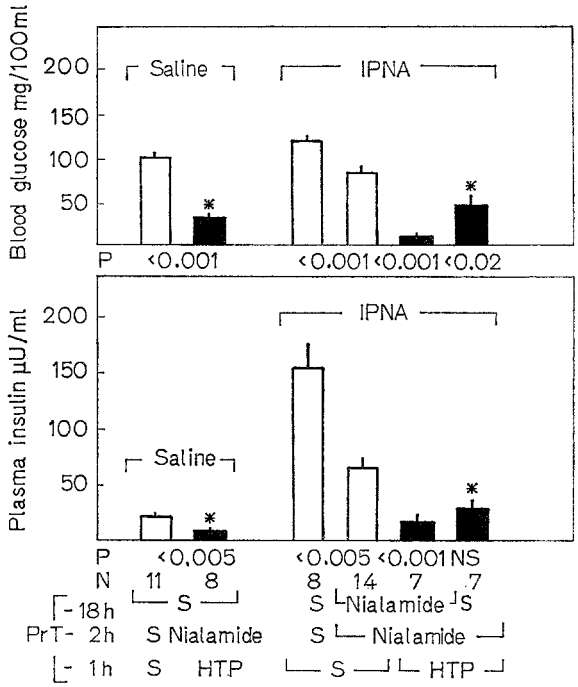

Fig.3. Blood glucose levels and immunoreactive plasma insulin levels after intravenous injection of saline and *L*-IPNA (1.37 µmol/kg), respectively. Pretreatment with saline (S) or nialamide (0.27 mmol/kg) was given by two injections −18, and −2 h before the experiment. *L*-5-HTP, 0.26 mmol/kg (black columns) or saline (white columns) was given one hour prior to the experiment. Columns marked by an asterisk (*) indicate groups of animals pretreated with only one injection of nialamide (−2 h) and a small dose of *L*-5-HTP (0.03 mmol/kg). Vertical bars indicate standard error of the mean. *P* = probability level of random difference. NS = not significant. N = number of animals in each group. PrT = pretreatment. S = saline. HTP = *L*-5-HTP

The effect of an inhibitor of aromatic amino acid decarboxylase, Ro-4-4602, was studied on glibenclamide-stimulated insulin secretion since glibenclamide-induced insulin release was markedly inhibited by a previous injection of *L*-5-HTP (Fig. 1). Fig. 4b shows the results. It appears that Ro-4-4602 (administered −2 h) had no influence on basal insulin secretion. It is also evident that the inhibitory action of a large dose of *L*-5-HTP (0.26 mmol/kg administered one hour after Ro-4-4602) on glibenclamide-induced insulin release (Fig. 1) was completely abolished.

Acute changes in the level of blood glucose in normal mice following administration of monoamine oxidase inhibitors with or without L-5-HTP and 5-HT

Groups of normal non-fasted mice (5—7 animals per group) were injected intraperitoneally with 0.9% NaCl or nialamide (0.27 mmol/kg). Blood samples were taken at 0.30 and 60 min. Immediately following the blood sample at 60 min, the animals were injected intravenously with either saline or *L*-5-HTP (0.07 mmol/kg) and the pattern of the blood glucose response was recorded as illustrated in Fig. 5 (mean values of each group are indicated). Fig. 5 shows that a single injection of nialamide or *L*-5-HTP had no acute effect on the blood glucose level compared with saline-injected controls. The combined treatment of nialamide and *L*-5-HTP, however, induced a marked hypoglycaemia lasting for at least 4 h.

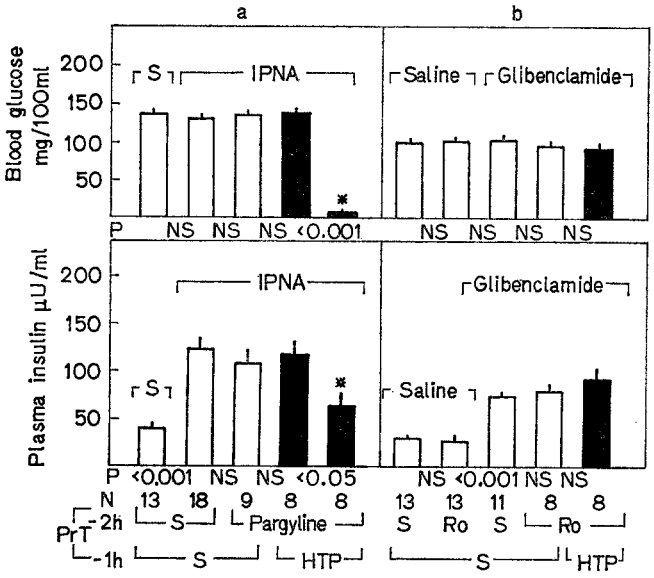

Fig. 4. a) Blood glucose levels and immunoreactive plasma insulin levels after intravenous injection of saline or *L*-IPNA (1.37 µmol/kg), respectively. Pretreatment with saline (S) or pargyline (0.27 mmol/kg) was given by one injection (−2 h) before the experiment. *L*-5-HTP, 0.03 mmol/kg (black columns) or saline (white columns) was given one hour prior to the experiment. Columns marked by an asterisk (*) indicate a group of animals pretreated with three injections of pargyline (−25, −18, and −2 h) and a large dose of *L*-5-HTP, (0.26 mmol/kg). Pargyline injections at −25, and −18 h are not denoted in the pretreatment scheme below the figure

b) Blood glucose levels and immunoreactive plasma insulin levels after intravenous injection of saline or glibenclamide (0.5 µmol/kg), respectively. Pretreatment with saline (S) or Ro-4-4602 (Ro), 0.85 mmol/kg, was given by one injection (−2 h) before the experiment. *L*-5-HTP, 0.26 mmol/kg (black columns), or saline (white columns) was given one hour prior to the experiment. Vertical bars indicate standard error of the mean. *P* = probability level of random difference. NS = not significant. N = number of animals in each group. PrT = pretreatment. S = saline. HTP = *L*-5-HTP

The same type of experiment was repeated using the non-hydrazine monoamine oxidase inhibitor, pargyline (0.27 mmol/kg). At 60 min the animals were injected intravenously with saline, *L*-5-HTP (0.07 mmol/kg) or 5-HT (0.26 mmol/kg). In addition, one group of mice received the decarboxylase inhibitor Ro-4-4602 (0.85 mmol/kg) 15 min before administration of pargyline. At 60 min this latter group was injected with a large dose of *L*-5-HTP (0.26 mmol/kg). Fig. 6 illustrates the results. From Fig. 6 it appears that the combined treatment of pargyline and *L*-5-HTP induced a slight hypoglycaemia compared with the control group injected with pargyline-saline. This hypoglycaemia was abolished after pretreatment with Ro-4-4602, in spite of the large dose of *L*-5-HTP used in this group, and changed into a slight hyperglycaemia. 5-HT and especially the combined treatment with pargyline and 5-HT, induced a marked hyperglycaemia.

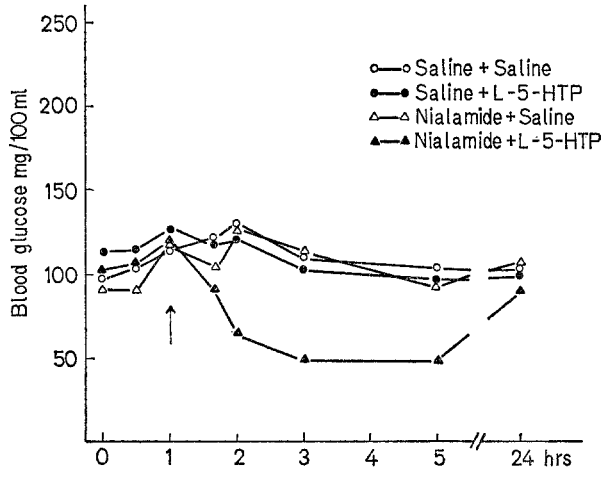

Fig. 5. Acute changes in the level of blood glucose in normal, non-fasted mice following the intravenous injection of saline or *L*-5-HTP, 0.07 mmol/kg (marked by an arrow). Saline or nialamide, 0.27 mmol/kg was given intraperitoneally at the time 0. Each group consisted of 5—7 animals. Abscissa: time in hours. Ordinate: blood glucose level, mg/100 ml

Acute changes in the level of blood glucose in alloxan-diabetic mice following pargyline and L-5-HTP

Groups of alloxan-diabetic, non-fasted mice (6—10 animals per group) were treated with pargyline (0.27 mmol/kg) and *L*-5-HTP (0.26 mmol/kg) as described above for normal mice. Fig. 7 shows the results. There was no effect of pargyline alone or of *L*-5-HTP alone on the level of blood glucose compared with saline-injected controls. The combined treatment of pargyline and *L*-5-HTP elicited a profound hypoglycaemia. The alloxan-diabetic mice were very susceptible to this treatment, and several mice died in convulsions during the course of the experiments. The

dose of *L*-5-HTP effective in normal mice, 0.07 mmol/kg, administered together with pargyline, had no effect on the level of blood glucose of the alloxan-diabetic animals (not indicated in Fig. 7).

Effect of combined treatment with pargyline and L-5-HTP on tissue glycogen stores

Groups of normal, non-fasted mice were injected with either saline or the combined treatment of pargyline and *L*-5-HTP (Table 1). Intravenous injection of *L*-5-HTP (0.26 mmol/kg) was performed one hour after administration of pargyline. After another hour, the animals were killed and blood glucose, liver glycogen, and muscle (gastrocnemius) glycogen were determined. From Table 1 it appears that the profound hypoglycaemia recorded at this time was accompanied by virtual exhaustion of liver glycogen levels, and markedly decreased muscle glycogen levels. Test for urinary glucose by means of Clinistix®, 30 min after injection of *L*-5-HTP yielded negative result in 100%, although one of the control animals showed traces of glucose.

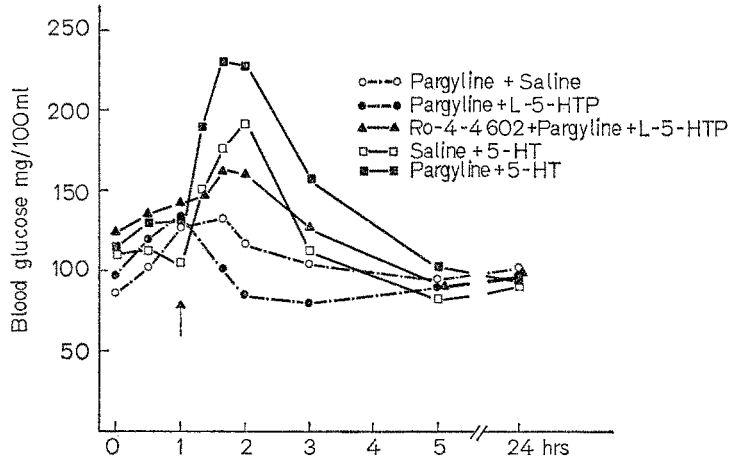

Fig. 6. Acute changes in the level of blood glucose in normal, non-fasted mice following the intravenous injection of saline, *L*-5-HTP, (0.07 mmol/kg), and 5-HT (0.26 mmol/kg), respectively (marked by an arrow). Saline or pargyline (0.27 mmol/kg) was given intraperitoneally at the time 0. One group of animals received Ro-4-4602, intraperitoneally (0.85 mmol/kg) at −15 min. Each group of animals consisted of 5—7 mice. Abscissa: time in hours. Ordinate: blood glucose level, mg/100 ml

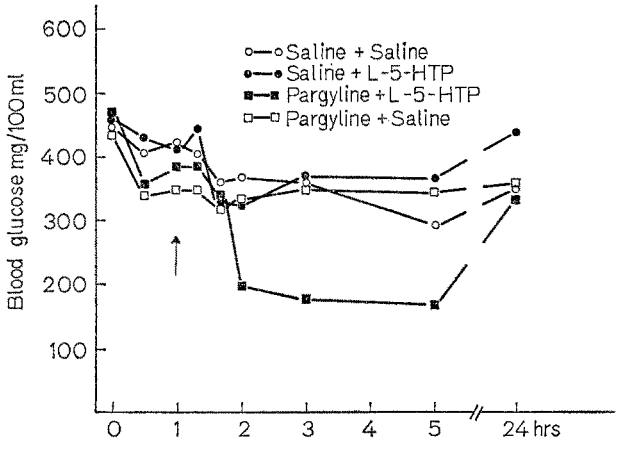

Fig. 7. Acute changes in the level of blood glucose in alloxan-diabetic, non-fasted mice following the intravenous injection of saline or *L*-5-HTP, 0.26 mmol/kg (marked by an arrow). At the time 0, saline or pargyline (0.27 mmol/kg) was given intraperitoneally. Each group consisted of 6—10 animals. Abscissa: time in hours. Ordinate: blood glucose level, mg/100 ml

Table 1. *Effect of the combined treatment of pargyline and L-5-HTP on the levels of blood glucose and tissue glycogen in normal, non-fasted mice. Pargyline (0.27 mmol/kg) was given 2 h and L-5-HTP (0.26 mmol/kg) 1 h before killing the animals. Controls received saline. Mean values and standard error of the mean are given. Figures in parenthesis indicate number of animals. P = probability level of random difference*

	Control		Pargyline + *L*-5-HTP		*p*
Blood glucose mg/100ml	113±4	(8)	25±5	(9)	$p < 0.001$
Liver glycogen mg/g	46.39±4.20	(8)	0.21±0.02	(9)	$p < 0.001$
Muscle glycogen mg/g	2.30±0.34	(8)	0.83±0.23	(9)	$p < 0.005$

Discussion

The possible role of monoaminergic mechanisms in the regulation of insulin secretion has been paid a great deal of attention by several investigators during the last few years, since fluorescence-microscopic studies have revealed stores of certain monoamines (5-HT and dopamine) in the pancreatic islets of several mammalian species, including man [3]. Moreover, uptake and decarboxylation of *L*-5-HTP and L-dihydroxyphenyl-

alanine (*L*-DOPA) have been demonstrated in the pancreatic islets of species not normally containing monoamines demonstrable by the conventional fluorescence-microscopic technique [3]. The possible functional role of 5-HT for insulin-secreting mechanisms has recently been investigated by several workers on preparations *in vitro* from different species [7, 8, 25, 27]. Telib *et al.* [25] found that 5-HT stimulated insulin release independently of glucose, from rabbit pancreatic tissue. Incubation with 5-HT + glucose led to a slight decrease in glucose-induced insulin release. Ui [27] postulated that 5-HT stimulated insulin release in the intact rat on the basis of studies *in vitro* utilizing the rat diaphragm bioassay of insulin. Feldman and Lebowitz [7, 8] reported that 5-HT had a marked inhibitory action on both basal and glucose-mediated insulin release in the golden hamster *in vitro*. These studies *in vitro* were, however, mainly concerned with the effect of added, extracellular 5-HT on the insulin-releasing mechanisms, whereas our studies are concerned with intracellular effects of 5-HT. Therefore, the results described in the present paper, based on experiments *in vivo* on basal and stimulated insulin secretion in mice after injection of the amine precursor *L*-5-HTP, with or without previous treatment with monoamine oxidase inhibitors, strongly suggest that *intracellular* levels of 5-HT (or possibly other related monoamines) have an important role *in vivo* as regulators of insulin secretion. This does not exclude the possibility that also extracellular levels of 5-HT may influence insulin secretion.

In a preceding paper [6] it was demonstrated by means of electron-microscopic autoradiography after injection of labelled 5-HTP, that the storage of intracellular 5-HT in the B cells is mainly confined to the specific granules also known to contain insulin or insulin precursors. The emiocytosis theory [14, 30] suggests that in response to glucose or sulphonylurea stimulation, the secretion granules migrate to the cell surface and fuse with the cell membrane whereby the secretion granules dissolve, and the soluble insulin enters the vascular compartment. These observations were described as occurring 10—15 min after tolbutamide administration. The timing of the morphologic events does not, however, coincide with the rapid insulin release (plasma insulin peaks are reached 1—3 min after intravenous administration of glucose or sulphonylurea). In recent ultrastructural studies of the B cell, in an attempt to correlate early morphological events to the initial dynamics of insulin secretion, it has been stated that glucose infusion in a preparation of pancreatic tissue *in vitro* elicits emiocytosis already after 1 min [15]. No emiocytosis was noted after tolbutamide. However, calculations based on the rate of the release of insulin and the insulin content of the pancreas indicate that the chance of visualizing granule extrusion by electron microscopy is small [9]. Total insulin secreted during the initial phase of secretion amounts to only 1—3% of the total insulin content of the intact pancreas [11]. Therefore, the absence of morphologic observations of rapid emiocytosis after sulphonylurea stimulation, does not invalidate our assumption that monoamine stores confined to the secretion granules may modify insulin release after stimulation by glibenclamide or *L*-IPNA. Moreover, as proposed by Orci *et al.* [18], insulin release induced by sulphonylurea does not occur through emiocytosis, but may result from intracellular liberation of the intragranular insulin stores by gradual dissolution of the hormone within the sac, followed by transfer through the cell without disruption of the granule or cell membranes. It is also assumed that insulin can be excreted rapidly from the cell by by-passing the secretion granules [16, 20]. Although the 5-HT stores are calculated to be at least 5—10 times higher in the specific secretion granules than in the remaining part of the B cell [6], monoaminergic influence on this alternative secretion process also cannot be excluded.

It is evident from Fig. 1 that glucose-mediated insulin release was not affected by raising the intracellular levels of 5-HT by a preceding intravenous dose of *L*-5-HTP. Neither did animals pretreated with the monoamine oxidase inhibitor nialamide, display any change in glucose-induced insulin release. In spite of the profound hypoglycaemia (mean blood glucose level 19 mg/100 ml) elicited after the combined treatment with nialamide and *L*-5-HTP, there was no effect on acute secretion of insulin after intravenous glucose load compared with the control group (Fig. 2). Acute release of insulin following the injection of the sulphonylurea compound glibenclamide, however, was significantly inhibited (mean increment in plasma insulin level was only about 40% of that of the saline-pretreated controls) by raised levels of intracellular amine elicited after exogenously administered *L*-5-HTP (Fig. 1) or after monoamine oxidase inhibition (Fig. 2). The abolition of the inhibitory influence of injected *L*-5-HTP on glibenclamide-mediated insulin secretion by an inhibitor of aromatic amino acid decarboxylase (Fig. 4), confirmed our assumption that intracellular levels of the amine and not of the amino acid are of decisive importance. The combined treatment with *L*-5-HTP and nialamide (Fig. 2) did not further decrease the insulin-releasing effect of glibenclamide, in spite of the additional condition of profound hypoglycaemia elicited by this treatment compared with animals treated with nialamide alone. This finding may indicate that part of the insulin pool that can be released by glibenclamide is not affected by either 5-HT or acute hypoglycaemia, thus resembling the acute insulin response following glucose, which was apparently totally unaffected. It is of particular interest that insulin release following glucose and glibenclamide, respectively, is not influenced by the marked hypoglycaemia which had lasted for about half an hour before the injection of the insulin-releasing agents. It must be emphasized that the results are obtained

during a non-insulin hypoglycaemia, and therefore are unaffected by the possible negative "feed back" of high concentrations of plasma insulin. The finding that insulin release mediated by glibenclamide but not by glucose was influenced by intracellular 5-HT indicates to some extent a different mode of action of these agents. However, another monoamine (dopamine) administered as *L*-DOPA in equimolar doses to *L*-5-HTP, has been shown to inhibit the insulin secretion mediated both by glibenclamide and by glucose (this laboratory, to be published), suggesting a possible quantitative or qualitative difference between the inhibitory action of these two monoamines on insulin secretion.

Insulin release induced by *L*-IPNA is slower in onset than insulin release following glucose or sulphonylurea. An inhibitory action of 5-HT was recorded both after *L*-5-HTP (Fig. 1) and after nialamide (Fig. 3). After the combined treatment of these agents, the insulin-releasing effect of *L*-IPNA was totally suppressed, which is in contrast to glucose- and glibenclamide-mediated insulin secretion. Thus the total pool of releasable insulin that can be mobilized by an acute stimulation of the beta-adrenergic monoamine IPNA, seems to be under the potential influence of intracellular levels of another monoamine (5-HT). It is worth noting that one injection of nialamide plus a small dose of *L*-5-HTP (Fig. 3) was equipotent with three injections of pargyline plus the larger dose of *L*-5-HTP (Fig. 4) in inducing a total suppression of insulin release mediated by *L*-IPNA. Whether this discrepancy is due to some "side-effect" of the hydrazine compound nialamide, remains to be elucidated. However, from the experience of the authors, it is recommended to treat the animals with two injections of nialamide (−18, and −2 h) or three injections of pargyline (−24, −18, and −2 h) to obtain a safe monoamine oxidase inhibition with regard to the B cells.

The influence of *L*-5-HTP and monoamine oxidase inhibitors on *basal* insulin secretion was not consistent. A large dose of *L*-5-HTP alone and 2 injections of nialamide plus *L*-5-HTP had no effect (Figs. 1 and 2), whereas 2 injections of nialamide alone (Fig. 2) and one injection of nialamide plus a small dose of *L*-5-HTP (Fig. 3) significantly decreased the basal insulin secretion. These results indicate that there is a possible inhibitory action of 5-HT on basal insulin secretion not manifested, however, after a large dose of exogenously administered *L*-5-HTP. Since certain amino acids are known to promote insulin secretion, it is not improbable that the inhibitory action of the amine 5-HT may be masked by an insulinogenic effect of the amino acid *L*-5-HTP.

In recent years several investigators have reported on the hypoglycaemic effect of a number of hydrazine and non-hydrazine monoamine oxidase inhibitors in man and animals, especially manifested after combination of a monoamine oxidase inhibitor and insulin or sulphonylurea [1,2, 4,5, 29]. The mechanism of the hypoglycaemic action of monoamine oxidase inhibitors is unknown, but is has been suggested that the monoamine oxidase inhibition might result in a replacement of adrenaline and noradrenaline in tissues by less potent adrenergic amines e.g. octopamine. This could then result in an impaired capacity of the adrenergic system to respond to the fall in blood glucose after insulin or sulphonylurea drugs [2, 5].

From the results presented in the present paper, it is evident that two injections of the hydrazine compound nialamide within 24 h, result in a slight but significant hypoglycaemia in normal, non-fasted mice (Figs. 2 and 3). The administration of *L*-5-HTP alone did not induce any acute significant change in blood glucose level, but after a previous dose of a monoamine oxidase inhibitor there was an invariable fall in the concentration of blood glucose. The combined treatment of nialamide and a small dose of *L*-5-HTP elicited a more pronounced fall in the level of blood glucose than did the treatment with equimolar doses of the non-hydrazine compound pargyline and *L*-5-HTP (Figs. 5 and 6). The mean fall in blood glucose compared with that in the control animals was −63 mg/100 ml and −64 mg/100 ml at 2 and 3 h, respectively, (nialamide); whereas the comparable values for pargyline-pretreated animals were −32 mg/100 ml and −23 mg/100 ml. The discrepancy may be explained by the inhibitory effect of hydrazine compounds on gluconeogenesis [19], and does not necessarily imply that pargyline *per se* is a weaker monoamine oxidase inhibitor than nialamide in this respect. The inability of *L*-5-HTP itself to lower the level of blood glucose was further demonstrated by inhibiting the decarboxylation of the amino acid by means of the decarboxylase inhibitor Ro-4-4602 (Fig. 6). The results indicate so far that neither the amino acid nor any metabolite of 5-HT is necessary to obtain the hypoglycaemic effect.

Acute treatment with monoamine oxidase inhibitors and a large dose of *L*-5-HTP resulted in an immediate (within 30 min) and profound hypoglycaemia in normal, non-fasted mice, without any measurable increment in insulin secretion. Moreover, alloxan-diabetic animals reacted with a marked decrease in blood glucose level, further corroborating that the phenomenon is independent of insulin action. Release of stored amines resulting in increased circulating levels of 5-HT (or dopamine) is not a probable explanation for the hypoglycaemic condition. On the contrary, the administration of an equimolar dose of 5-HT (Fig. 6) resulted in a marked rise in the blood glucose level, which was further augmented after the combination of pargyline and 5-HT. Furthermore, the administration of a monoamine oxidase inhibitor and *L*-DOPA induced a hyperglycaemic condition (13, and preliminary data from this laboratory). Increased carbohydrate storage could also be excluded as an explanation of the profound hypoglycaemic condition since glycogen levels in liver and skeletal muscle of

animals treated with pargyline and *L*-5-HTP were virtually exhausted (Table 1) without any sign of relieving the hypoglycaemia. Moreover, no increase in urinary glucose was recorded, rendering an augmented glucose elimination through the kidneys less probable. Increased peripheral glucose utilization and combustion seem to be the only reasonable explanation of the hypoglycaemic condition. This might justify the hypothesis that *intracellular levels of 5-HT* are able to influence glycogen mobilization and peripheral glucose utilization either by a direct action due to altered 5-HT levels in the peripheral cell itself, or, not excluded, through an indirect action, e.g. as a consequence of altered 5-HT levels in the central nervous system. Therefore, we do not think that the mechanism of the hypoglycaemic action of monoamine oxidase inhibitors is the result primarily of a replacement of catecholamine stores in tissues by biologically less potent amines such as octopamine, but rather of raised intracellular levels of 5-HT. Moreover, it cannot be excluded that intracellular levels of 5-HT may influence insulin secreting mechanisms through effects on the glycogen stores in the pancreatic B cells. So far, however, the results presented in this paper suggest that intracellular 5-HT levels in the pancreatic B cells possess the ability to modify insulin secretion *in vivo* chiefly by an inhibitory action on insulin release following raised intracellular concentrations of the amine.

References

1. Adnitt, P.I.: Hypoglycemic action of monoamineoxidase inhibitors (MAOI'S). Diabetes **17**, 628—633 (1968).
2. Barrett, A.M.: The mode of action of insulin potentiation by mebanazine. J. pharm. Pharmac. **22**, 291—297 (1970).
3. Cegrell, L.: The occurrence of biogenic monoamines in the mammalian endocrine pancreas. Acta physiol. scand. Suppl. **314**, 1—60 (1968).
4. Cooper, A.J., Ashcroft, G.: Potentiation of insulin hypoglycaemia by M.A.O.I. antidepressant drugs. Lancet **1966 I**, 407—409.
5. — Modification of insulin and sulfonylurea hypoglycemia by monoamine-oxidase inhibitor drugs. Diabetes **16**, 272—274 (1967).
6. Ekholm, R., Ericson, L.E., Lundquist, I.: Monoamines in the pancreatic islets of the mouse. Subcellular localization of 5-hydroxytryptamine by electron microscopic autoradiography. Diabetologia **7**, 339—348 (1971).
7. Feldman, J.M., Lebowitz, H.E.: Specificity of serotonin inhibition of insulin release from golden hamster pancreas. Diabetes **19**, 475—479 (1970).
8. — Serotonin inhibition of *in vitro* insulin release from golden hamster pancreas. Endocrinology **86**, 66—70 (1970).
9. Findlay, J.A., Gill, J.R., Irvine, G., Lever, J.D., Randle, P.J.: Cytology of β-cells in rabbit pancreas pieces incubated *in vitro*; Effects of glucose and tolbutamide. Diabetologia **4**, 150—160 (1968).
10. Gershon, M.D., Ross, L.L.: Location of sites of 5-hydroxytryptamine storage and metabolism by radioautography. J. Physiol. (Lond.) **186**, 477—492 (1966).
11. Grodsky, G., Landahl, H., Curry, D., Bennett, L.: *In vitro* studies suggesting a two-compartmental model for insulin secretion. In: Falkmer, S., Hellman, B., Täljedal, I.-B. (Eds): The structure and metabolism of the pancreatic islets, pp. 409—421. Oxford: Pergamon Press 1970.
12. Heding, L.: A simplified insulin radioimmunoassay method. In: Donato L. et al. (Eds): Labelled proteins in tracer studies, pp. 345—350. Brussels: Euratom 1966.
13. Håkanson, R., Lundquist, I., Rerup, C.: On the hyperglycaemic effect of DOPA and dopamine. Europ. J. Pharmacol. **1**, 114—119 (1967).
14. Lacy, P.E.: The pancreatic beta cell. Structure and function. New Engl. J. Med. **276**, 187—194 (1967).
15. Lee, J.C., Grodsky, G.M., Bennett, L.L., Smith-Kyle, D.F., Craw, L.: Ultrastructure of β-cells during the dynamic response to glucose and tolbutamide *in vitro*. Diabetologia **6**, 542—549 (1970).
16. Like, A.A., Miki, E.: Diabetic syndrome in sand rats. IV. Morphologic changes in islet tissue. Diabetologia **3**, 143—166 (1967).
17. Marks, V.: An improved glucose oxidase method for determining blood, C.S.F. and urine glucose levels. Clin. Chim. Acta **4**, 395—401 (1959).
18. Orci, L., Stauffacher, W., Beaven, D., Lambert, A.E., Renold, A.E., Rouiller, C.: Ultrastructural events associated with the action of tolbutamide and glybenclamide on pancreatic β-cells *in vivo* and *in vitro*. Acta Diabet. Lat. **6**, Suppl. 1, 271—374 (1969).
19. Ray, P.D., Hanson, R.L., Lardy, H.A.: Inhibition by hydrazine of gluconeogenesis in the rat. J. biol. Chem. **245**, 690—696 (1970).
20. Renold, A.E.: Insulin biosynthesis and secretion — a still unsettled topic. New Engl. J. Med. **282**, 173—182 (1970).
21. Rerup, C., Lundquist, I.: Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling. Acta endocr. (Kbh.) **52**, 357—367 (1966).
22. — Blood glucose level in mice. 2. A quantitative study of alloxan diabetes. Acta endocr. (Kbh.) **54**, 514—525 (1967).
23. — Non-specific reaction of current glucose oxidase preparations with glycogen and its application for glycogen determinations in tissues. Acta pharmacol. (Kbh.) **25**, 41—53 (1967).
24. Ritzén, M., Hammarström, L., Ullberg, S.: Autoradiographic distribution of 5-hydroxytryptamine and 5-hydroxytryptophan in the mouse. Biochem. Pharmacol. **14**, 313—321 (1965).
25. Telib, M., Raptis, S., Schröder, K.E., Pfeiffer, E.F.: Serotonin and insulin release *in vitro*. Diabetologia **4**, 253—256 (1968).
26. Udenfriend, S., Weissbach, H., Bogdanski, D.F.: Increase in tissue serotonin following administration of its precursor 5-hydroxytryptophan. J. biol. Chem. **224**, 803—810 (1957).
27. Ui, M.: The role of serotonin in carbohydrate metabolism. VII. *In vitro* observations suggesting enhanced release of insulin in response to serotonin treatment. Endocrinol. Japon. **9**, 22—32 (1962).
28. Underhill, F.P.: Studies in carbohydrate metabolism. I. The influence of hydrazine upon the organism, with special reference to the bood sugar content. J. biol. Chem. **10**, 159—168 (1911).
29. Wickström, L., Pettersson, K.: Treatment of diabetics with monoamine-oxidase inhibitors. Lancet **1964 II**, 995—997.
30. Williamson, J.R., Lacy, P.E., Grisham, J.W.: Ultrastructural changes in islets of the rat produced by tolbutamide. Diabetes **10**, 460—469 (1961).

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