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Serotonin and Insulin Release in vitro

M. Telib, S. Raptis, K.E. Schröder and E.F. Pfeiffer*

Department of Clinical Endocrinology and Metabolism, Centre of Medicine, University of Ulm/Donau

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Summary. Serotonin stimulated insulin release, independently of glucose, from rabbit pancreatic tissue *in vitro*. A concentration of 100 µg/ml gave a maximal insulin release. Higher concentrations delayed insulin release. — The simultaneous incubation with serotonin and glucose led to a slight decrease in insulin release. This decrease in insulin release rose with increasing serotonin concentrations. — The insulin production induced by serotonin stimulation (100 µg/ml) was quantitatively equivalent to the maximal amount of insulin released by 2 mg glucose per ml, and by 1 mg of tolbutamide per ml. — The significance of these findings with regard to the difference in the results obtained with the oral as compared with the intravenous glucose tolerance test, and with respect to the pathogenesis of both the dumping and the carcinoid syndromes were discussed.

Sérotonine et libération d'insuline in vitro

Résumé. La sérotonine stimule la libération de l'insuline par le pancréas du lapin *in vitro* indépendamment du taux du glucose dans le milieu d'incubation. La concentration de 100 µg/ml provoqua une sécrétion insulinique maximale. Des concentrations plus élevées inhibaient la sécrétion d'insuline. L'incubation simultanée avec sérotonine et glucose causa une diminution légère de la sécrétion insulinique. Cette diminution augmentait quand la concentration de sérotonine augmentait. L'effet de 100 µg/ml de sérotonine était comparable à l'effet de 2 mg/ml de glucose ou de 1 mg/ml de tolbutamide. La signification de ces observations en vue d'expliquer la différence entre les résultats obtenus avec le test de tolérance au glucose par voie orale et par voie intraveineuse a été discutée ainsi que leur signification dans la pathogénie du dumping-syndrome et du syndrome du carcinoïde.

Serotonin und Insulinfreisetzung in vitro

Zusammenfassung. Serotonin stimuliert die Insulinfreisetzung aus dem Kaninchenpankreas *in vitro* unabhängig vom Glucosespiegel im Inkubationsmedium. Die Konzentration von 100 µg/ml führte zu einer maximalen Insulinausschüttung. Höhere Konzentrationen hemmten die Freisetzung des Insulins. Die gleichzeitige Inkubation mit Serotonin und Glucose rief eine geringfügige Verminderung der Insulinsekretion hervor. Diese Verminderung nahm mit steigender Serotoninkonzentration zu. — Die nach Stimulierung mit Serotonin (100 µg/ml) ausgeschüttete Insulinmenge entsprach der nach Zusatz von 2 mg Glucose/ml und 1 mg Tolbutamid/ml sezernierten. Die Bedeutung dieser Befunde für das Verständnis der unterschiedlichen Ergebnisse nach oraler und intravenöser Glucosebelastung wurde ebenso diskutiert wie ihre Bedeutung für die Pathogenese von Dumping- und Karzinoid-Syndrom.

Key-words: Serotonin, insulin secretion in vitro, Carcinoid, Dumping.

The insulin concentration in the serum following a glucose load in a tolerance test is higher when the glucose is given orally than when it is given intravenously, and this difference is attributed to the presence of insulin-releasing substances in the mucous membrane of the gastro-intestinal tract. Among these are secretin (Mc Intyre et al., 1965; Pfeiffer et al., 1965; Bottermann et al., 1967a and b; Raptis et al., 1967) glucagon and pancreozymin (Langs and Friedberg, 1965; Samols et al., 1965; Meade et al., 1967; Schröder et al., 1967; Unger et al., 1967).

Serotonin is present in abundant amounts in the small intestine, in particular in the duodenum and the jejunum (Erspamer, 1953, 1954, Tobe et al., 1966); and it is involved in carbohydrate metabolism (Kobayashi et al., 1960); thus raising the question whether it may belong to these gastro-intestinal hormones that stimulate insulin release. A glycogenetic effect of serotonin, discussed by Ui in 1962 was attributed to serotonin-mediated secretion of endogenous pancreatic insulin.

The present paper deals with the effect of serotonin on the insulin secretion of isolated pancreatic tissue, continuing and extending the first communication of one of us (Pfeiffer) to the VI Congr. Intern. Diab. Fed., Stockholm 1967.

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Method

Part of the method has been published elsewhere (Telib et al., 1966). Male rabbits weighing about 2—3 kg and fasted overnight, were killed with sodium barbitol; the pancreas was immediately removed, placed in cold physiologic saline (0.9 gNaCl/100 ml), and cleaned of all blood vessels and fat. The thick portion of the caudal part of the pancreas was cut with a Stadie-Riggs Microtome to give a thin layer of tissue of about 0.5 mm. The pancreatic pieces were incubated in 4 ml of Krebs-Ringer bicarbonate, supplemented with the sodium salts of pyruvic, glutamic and fumaric acids (Coore and Randle, 1962, 1964; Telib, 1968). After a preincubation period of 30 min, the tissue was incubated in buffer with the following constitution:

1. buffer alone for measuring basic secretion;
2. buffer with a glucose concentration of 200 mg%;
3. buffer with tolbutamide as a comparative stimulant;
4. buffer with varying amounts of 5-hydroxytryptamine (serotonin).

After the incubation period, the insulin content of the medium was measured with the rapid radio-immunological method (TELIB and PFEIFFER, 1968).

Results

Fig. 1 shows the effect of the different serotonin concentrations on insulin release *in vitro*. A concentration of 10 μg/ml gave a significant rise in insulin release over the basal value; i.e. 440 ± 32 μU of insulin per ml with buffer alone, and 574 ± 55 μU/ml with 10 μg of serotonin per ml. Increasing the concentration of serotonin led to no further significant increase in insulin secretion. Although a 10-fold increase in serotonin concentration gave a significantly higher increase in insulin release, raising the concentration to 500 μg/ml led not only to no further increase, but in fact gave a significant inhibition of insulin release. Expressed as percent of the effect with the buffer alone (basal value, 100%), the 10 μg/ml concentration gave an increase 130%; the 100 μg/ml, 200%; with 200 μg/ml there was a drop to 105%, and with 500 μg/ml a further drop to 75%. The combination of glucose (200 mg%) and serotonin (Fig. 2) gave no additional effect on insulin release, and in fact there was a decrease in insulin release compared with glucose or serotonin alone. Fig. 3 illustrates the comparison between the effect of the optimal concentrations of serotonin, glucose 2 mg/ml and tolbutamide 1 mg/ml. The effect of 100 μg of serotonin per ml was more or less the same as that of 1 mg of tolbutamide.

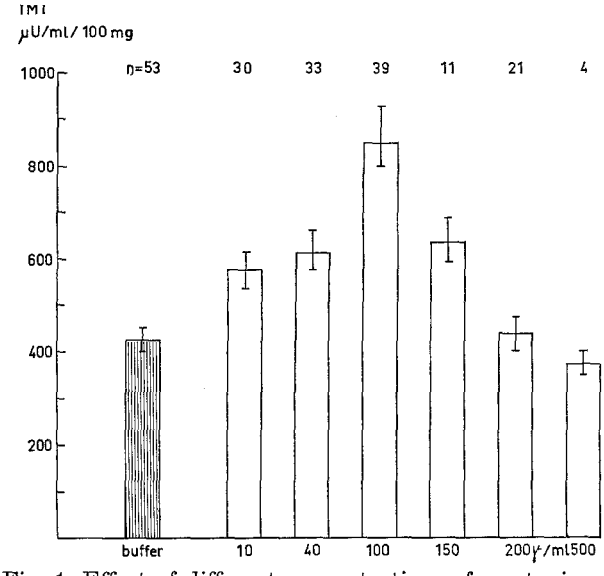

Fig. 1. Effect of different concentrations of serotonin on insulin release from rabbit pancreatic tissue *in vitro*

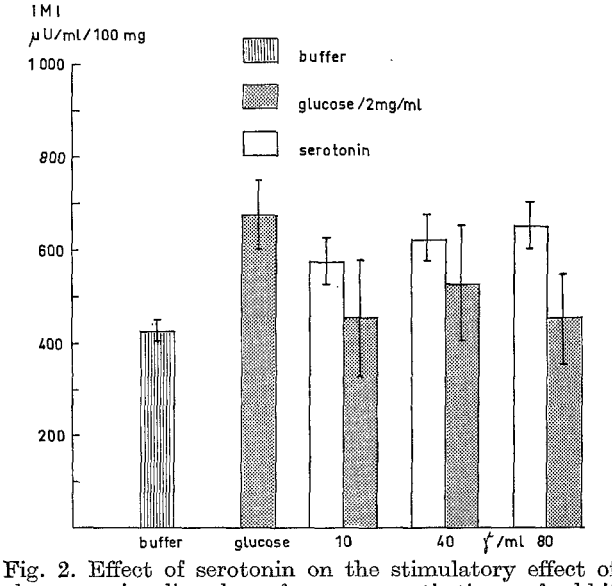

Fig. 2. Effect of serotonin on the stimulatory effect of glucose on insulin release from pancreatic tissue of rabbit *in vitro*

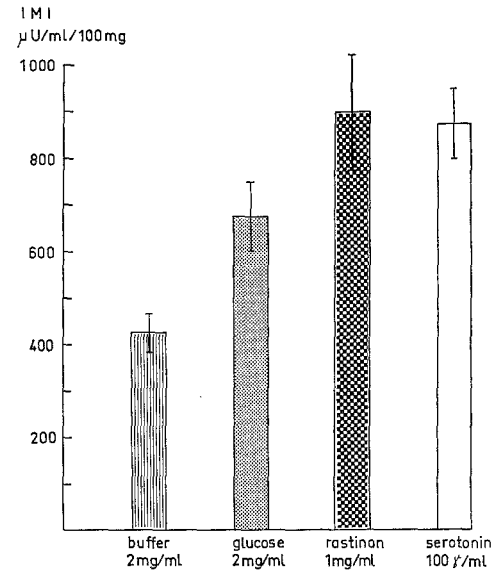

Fig. 3. Comparison of effect of serotonin (100 μg/ml) with that of glucose and rastinon on insulin release from rabbit pancreas *in vitro*

Discussion

The data presented here seem to verify the postulate of UI, (1962) that serotonin stimulates insulin secretion. This stimulating effect, as shown from our results *in vitro*, may be assumed to be a physiological one. WEST (1958) pointed out that pancreas possesses 5-hydroxytryptophan-decarboxylase activity, which is the enzyme forming serotonin from 5-hydroxytryptophan, and that monoamine oxidase activity, though found in pancreatic tissue as well (FALCK and HELLMANN, 1964) was much lower than 5-hydroxytryptophan decarboxylase. His finding appears to suggest the existence of serotonin in pancreatic tissue in fairly large amounts. Using a fluorescent microscopical method, FALCK and HELLMANN, (1964) and DAYAN

(1967) have shown that in several species, but not in man, the γ-cells of the islets of Langerhans contain 5-hydroxytryptamine. Besides its presence in the β-cells, it is abundant in the small intestine, especially the duodenum of many mammals (PENTILLÄ, 1966).

The fact that oral glucose tolerance leads to a higher insulin value in serum than does the intravenous one, is explained by many authors to be due to the presence of intestinal hormones secreted from the duodenal mucous membrane, which act either directly or indirectly on the pancreas. Some of these hormones have already been tested for insulin release *in vivo* (SAMOLS et al., 1965; SIMPSON et al., 1966; MELANI et al., 1967; PFEIFFER, 1967; RAPTIS et al., 1967; RAPTIS et al., 1968; UNGER et al., 1967) and *in vitro* (PFEIFFER et al., 1965; TELIB et al., 1966; SCHRÖDER et al., 1967; TURNER et al., 1966; TELIB 1968).

Another support for the serotonin-insulin relationship may be derived from the fact that after intravenous injection of growth hormone, serotonin is released in the pancreatico-duodenal vein (GALANSINO et al., 1963; SIREK et al., 1966); and after growth hormone injection, there is also a rise in insulin release (PFEIFFER et al., 1964; MELANI in our laboratory — unpublished data). Thus it may be, that the serotonin, which is released following the growth hormone injection, is responsible for the release of insulin.

The effect of serotonin on the blood sugar remains a matter of controversy. Hyperglycaemic (SIREK et al., 1957; COLOMBO et al., 1960; GALANSINO et al., 1960) as well as hypoglycaemic (MIRSKY et al., 1957; KOBYASHI et al., 1960) actions have been discussed. In support of the latter conclusion are the clinical symptoms which accompany both the dumping and the carcinoid syndromes. In both there is an elevation of 5-hydroxytryptamine in blood, and in most cases a marked fall in blood sugar was observed (DRAPANAS et al., 1962; PEART et al., 1963; VAN DER SLUYS VEER et al., 1964; ZEITLIN and SMITH, 1966). On the other hand after serotonin injection a rise in non-esterified fatty acids (NEFA) in serum was found (CARLSON et al., 1967), suggesting a lipolytic effect of serotonin *in vivo*. However this effect was not demonstrated *in vitro* (ITAYA and UI, 1964; BIECK et al., 1966). It is therefore possible, that serotonin, when administered *in vivo* may stimulate the mobilization of NEFA, not by a direct effect of its own on adipose tissue, but indirectly through the liberation of other hormones. For example, the liberation of epinephrine, which is responsible for the hyperglycaemia resulted after a hypoglycaemic phase due to insulin release in an intact rat, since no hyperglycaemia was observed in an adrenomedullated one (KOBAYASHI et al., 1960). The lipolytic effect of serotonin may not be seen under normal conditions due to the fact that the amine is rapidly metabolized in adipose tissue by monoaminoxidase. It is interesting to note that 3,5-AMP, which stimulates insulin release (WILLIAMS and ENSINCK, 1966), is formed from serotonin (BIECK et al., 1966).

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