Diabetologia 10, 411—414 (1974)

The Effect of Serotonin on in Vitro Insulin Secretion and Biosynthesis in Mice

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Received: May 16, 1973, and in revised form: May 6, 1974

Summary. The effect of serotonin on insulin secretion and biosynthesis was studied using isolated islets of mice. Serotonin produced a small stimulatory effect on insulin secretion when glucose was present in the incubation medium at a low concentration. On the other hand, an inhibition of insulin secretion was obtained with serotonin when glucose in the medium reached 3.0 mg/ml concentration. No significant effect of serotonin was obtained on insulin biosynthesis, neither in the presence of low nor with a high glucose concentration. These results suggest that the effect of this monoamine on insulin secretion is not mediated via its effect on insulin biosynthesis.

Key words: Insulin secretion, insulin biosynthesis, pancreatic monoamines.

Since Falck *et al.* described the presence of monoamines in the endocrine pancreas [3], several authors have been engaged in the study of these substances and their effect on insulin secretion [1, 2, 4—12, 14, 21, 22]. In this respect, serotonin has been one of the most extensively studied factors [4—6, 9, 12, 14—16, 21, 22) and it is now accepted that it plays a role in the control of insulin secretion. Its effect seems to depend on the glucose concentration and also on the species tested. Indeed, work already published shows that serotonin blocks the glucose-induced insulin release in the golden hamster [4—6], stimulates insulin secretion where glucose is present at a low concentration, as in rabbits [22] and rats [9], while in mice it inhibits [14—16] or shows no effect upon the secretion of insulin [4]. It is not as yet quite clear by which mechanism serotonin exerts the above mentioned effects. Furthermore there was no available report concerning the effects of this monoamine on insulin biosynthesis. Therefore, in view of the contradictory reports regarding the effect of serotonin in mice and the lack of information on insulin biosynthesis, we decided to study both processes in these animals.

Material and Methods

Normal Swiss albino male mice of about 30 g body weight were used. The animals were sacrificed by cervical dislocation and decapitation and the whole pancreas was quickly removed. The pancreata were digested with collagenase according to the method described by Lacy and Kostianovsky [13].

To study insulin secretion, 10 isolated islets were carefully selected and dropped into tubes containing 1 ml of Krebs Ringer bicarbonate buffer, pH 7.4, with bovine serum albumin 50 mg/ml, a protease inhibitor (Trasylol®, Bayer, 1000 KIU/ml) and glucose (0.6 or 3.0 mg/ml) with or without the addition of serotonin (0.1 mg/ml). This buffer was previously gassed with a mixture of O_2:CO_2 (95:5%) while, throughout the incubation period, a constant atmosphere of this gas mixture was maintained within the tubes. The incubation was carried out for 2 h at 37°C in a shaking incubator. During this period suitable aliquots for insulin determination were obtained at 0, 30, 60 and 120 min and immediately frozen until the hormone determination was performed. Immunologically measurable insulin (IMI) was determined following the procedure described by Melani *et al.* [18].

When studying insulin biosynthesis, 40 islets were incubated for 3 h in 1 ml of the same buffer as described above with the addition of 17 natural aminoacids (20 μg/ml of each amino acid, leucine excluded) and 50 μCi ^{3}H-L-leucine (19 Ci/mMol, Amersham). At the end of the incubation period, both the islets and the incubation medium were frozen together and thawed, homogenized, precipitated with 10% trichloroacetic acid and chromatographed through a G 50 fine Sephadex column. Fractions of 1 ml were collected and suitable aliquots were obtained for radioactivity assay in a liquid scintillation counter. Details of this procedure as well as the identification of the peaks obtained have already been published [20]. Briefly, this method enables one to identify a protein peak followed by a proinsulin, insulin and free-labelled leucine peak, respectively.

Results

Fig. 1 and 2 show the results of insulin secretion obtained with 0.6 mg/ml glucose and 3.0 mg/ml glucose respectively, alone or in the presence of serotonin. It can be seen that glucose at a low concentration elicited a small secretion of insulin at the end of the 2 h incubation period. Serotonin at this glucose concentration stimulated the release of insulin and/or enhanced

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** Supported by Deutsche Forschungsgemeinschaft Bonn-Bad Godesberg, SFB 87 Ulm

the effect of glucose. Serotonin showed no significant effect during the first 60 min of incubation. When the islets were incubated with a 3.0 mg/ml glucose concentration, a large release of insulin was already seen at 30 min of incubation. Serotonin in this instance significantly decreased the glucose-induced insulin release. This effect by serotonin, though already observed at 30 min, was most marked at 60 and 120 min.

Figs. 3 and 4 show the results obtained with insulin biosynthesis at 0.6 mg/ml and 3.0 mg/ml glucose concentration respectively, with or without the addition of serotonin. It can be seen that neither the 0.6 mg/ml glucose nor this glucose concentration plus serotonin produced a significant effect on insulin biosynthesis.

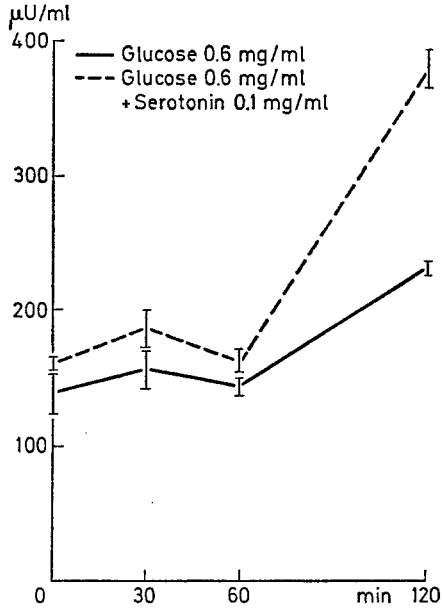


Fig. 1. shows the effect of glucose 0.6 mg/ml and glucose + serotonin on insulin secretion using 10 isolated islets per tube. Samples were obtained at 0, 30, 60 and 120 min of the incubation. Each point represents the average of 9 experiments ± S.E.M. The p-value between the control and serotonin at 120 min was < 0.05

5-hydroxytryptophan (5-HTP) [14]. This author found that the release of insulin in the presence of 0.6 mg/ml glucose was somewhat enhanced during and after preincubation with 5-HTP while no stimulation of insulin secretion was obtained when the islets were challenged by 3.0 mg/ml glucose. Other authors have already described a serotonin-induced insulin release in the presence of low glucose concentration

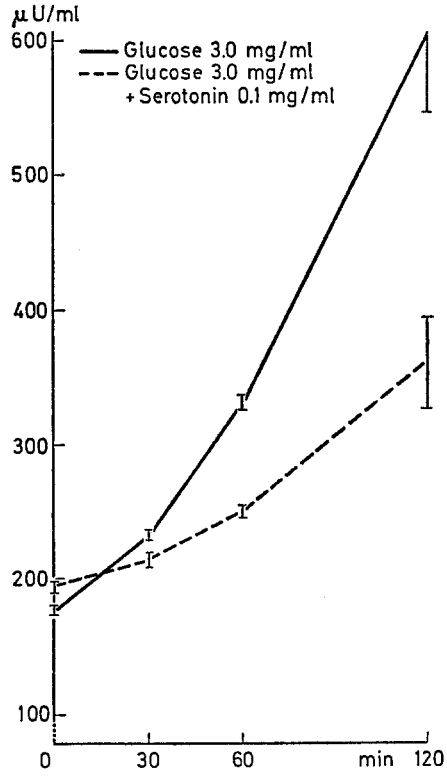


Fig. 2. represents the effect of glucose 3.0 mg/ml and glucose + serotonin on insulin secretion using 10 isolated islets per tube. Samples were obtained at 0, 30, 60 and 120 min of the incubation. Each point represents the average of 9 experiments ± S.E.M. The p-value between the control and serotonin at 60 and 120 min was < 0.01

Conversely, glucose in a 3.0 mg/ml concentration clearly stimulated the biosynthesis of proinsulin and insulin. No significant effect of serotonin on this glucose-induced proinsulin-insulin biosynthesis was observed.

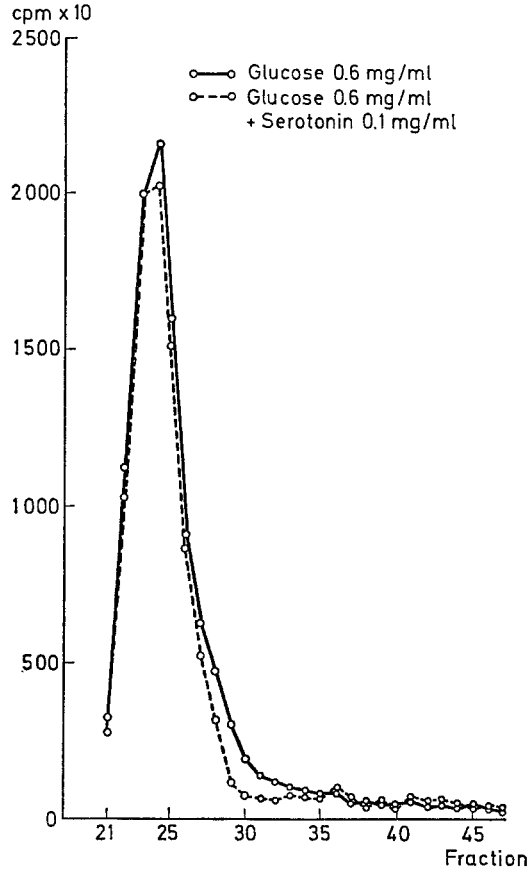
Discussion

Our results show clearly that in our strain of mice serotonin produced a small but significant stimulatory effect on insulin secretion when glucose was present in the incubation medium at a low concentration. Serotonin was observed to have the opposite effect when glucose in the medium reached 3.0 mg/ml concentration. Similar results were obtained by Lernmark with microdissected islets of obese-hyperglycemic mice preincubated with the natural serotonin precursor, in rabbits [22] and rats [9]. On the other hand, a biphasic effect on insulin secretion, depending on the glucose concentration, has already been described, using other substances like secretin [19]. It has to be stressed that, in our experiments, both effects of serotonin appeared most markedly during the subsequent 30 min incubation period. The report of Tamarit *et al.*, showing in the perfused rat pancreas that serotonin only affects the late phase of insulin secretion is in keeping with our findings. This fact could explain the lack of effect described by Feldman and co-workers with serotonin in mice [4], since they performed their experiments for a duration of only 15 min.

We were unable to demonstrate that serotonin had any significant effect on the biosynthesis of proinsulin-insulin, either in the presence of low or a high glucose concentration. On account of these

results, it could be suggested that the effect of serotonin on insulin secretion, at least in short term experiments, cannot be ascribed to its effect on the biosynthesis of insulin. This lack of action of serotonin on insulin biosynthesis, though indirectly, might suggest that this monoamine does not significantly modify glucose metabolism within the beta cells.

Thus, its effect might be due to a modification at the level of the glucose-membrane receptor, like the one described with alpha-neuraminidase [17]. An other possibility is that the increment of the serotonin within the beta granules [12] could modify the granules availability for emiocytosis.



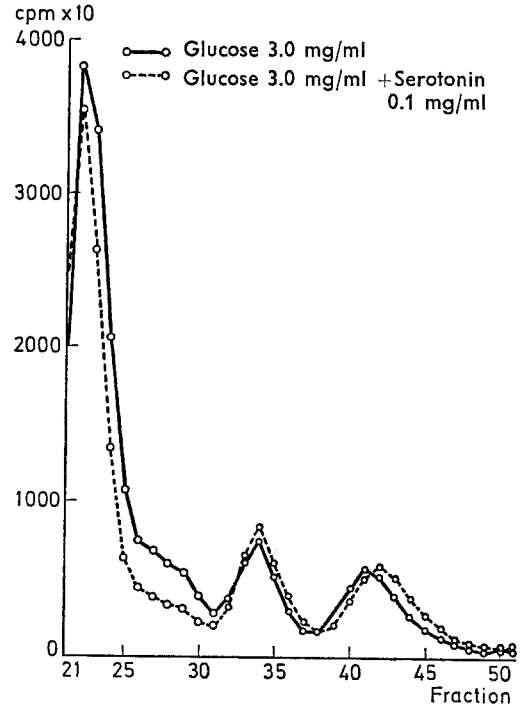


Fig. 3 and 4. The figures represent the incorporation of ^{3}H-leucine into proinsulin (second peak) and insulin (third peak) fraction of 40 isolated islets of mice after 3 h incubation, in the presence of 0.6 mg/ml and 3.0 mg/ml glucose with or without the addition of 0.1 mg/ml serotonin. The eluate fraction numbers are represented on the abscissa. The first peak eluting with the void volume represents islet proteins different from proinsulin and insulin. Each curve is the average of 6 experiments

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