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Effect of gentamycin on insulin release and ^{45}Ca net uptake by isolated islets[1]

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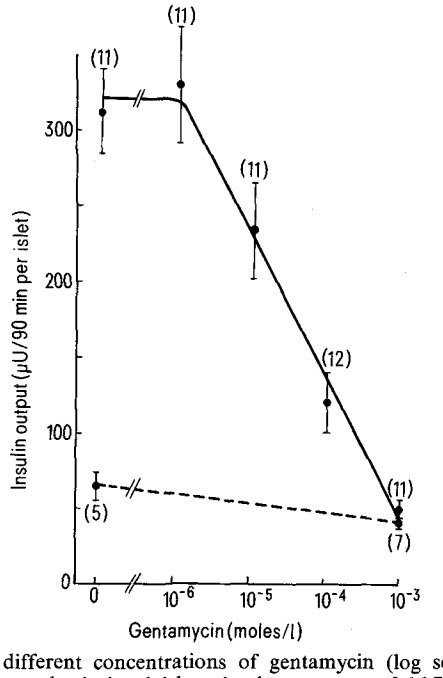
Summary. In isolated islets, gentamycin reduced both the ^{45}Ca net uptake and insulin release induced by glucose, but failed to inhibit the insulin secretion provoked by the combination of Ba^{2+} and theophylline. This indicates that the inhibitory effect of gentamycin is due to a reduction of Ca^{2+} entry into B-cells instead of to a harmful effect upon the integrity of the effector system, responsible for the extrusion of the insulin containing granules.

Recent reports have indicated that the inhibitory action of some aminoglycoside antibiotics on synaptic transmission, cardiovascular functions and smooth muscle contraction is related to their calcium antagonistic properties[4-6]. Because Ca^{2+} plays a crucial role in the mechanism of glucose-induced insulin release[7], this investigation was conducted to determine a possible inhibitory effect of gentamycin, a representative aminoglycoside antibiotic, upon the insulin release and ^{45}Ca uptake by isolated islets.

Materials and methods. The experiments were done with isolated islets obtained by the collagenase procedure[8] from pancreas of fed Wistar rats. The medium used in all experiments was a bicarbonate-buffered solution, enriched with 3 mg/ml of bovine serum albumin and equilibrated with a mixture of 95% O_2: 5% CO_2. In some experiments the medium was deprived of Ca^{2+} and enriched with Ba^{2+} and theophylline. The medium also contained, when required, 16.7 mM glucose. For insulin release, groups of 4 islets each were incubated at 37 °C under constant shaking in 1.0 ml of medium. After 90 min of incubation, an aliquot of the medium was drawn and stored at −20 °C for subsequent insulin dosage[9]. For measurement of ^{45}Ca net uptake, groups of 100 islets each were incubated for 90 min in the presence of ^{45}Ca (100 μCi/ml). The islets were then extensively washed with a nonradioactive medium and examined in sub-groups of 8 islets each for their ^{45}Ca content as described elsewhere[10]. All results were expressed as the mean (± SE) together with the number of individual experiments.

Results and discussion. Gentamycin inhibited the glucose-induced insulin release by isolated islets in a dose-related manner (fig.). In the 10^{-5} M range, gentamycin provoked a 24% reduction of the insulin release with respect to the control ($p < 0.05$). In the presence of 10^{-3} M gentamycin, the rate of glucose-induced insulin release was not significantly different from the basal value found in the absence of glucose. The ED_{50} for the inhibitory effect of this drug is close to 5.5×10^{-5} M. The figure also shows that 10^{-3} M gentamycin significantly reduced the insulin secretion in the absence of glucose ($p < 0.05$). According to Malaisse-Lagae and Malaisse[10] and Henquin and Lambert[11] there is a strict correlation between the insulin release and the ^{45}Ca net uptake by isolated islets. These autors showed that insulin secretion decreases more rapidly than the ^{45}Ca net uptake and that secretion is almost abolished when the ^{45}Ca net uptake is lowered below 60% of the control value, obtained in the presence of 16.7 mM glucose. Recently, Malaisse and others[12] suggested that the mechanism of insulin release requires the maintenance of an internally located Ca^{2+} pool which exhibits a high fractional turnover rate. Probably this pool is responsible for the above 60% Ca^{2+} threshold and corresponds to the so called La^{3+} nondisplaceable Ca^{2+} pool of Hellman et al[13]. In the presence of 16.7 mM glucose, gentamycin 10^{-4} and 10^{-3} M



Effect of different concentrations of gentamycin (log scale) on insulin release by isolated islets, in the presence of 16.7 mM of glucose (●——●) or in the absence of the sugar (●---●). Each point represents mean ± SE of the number of observations indicated in parentheses.

reduced the ^{45}Ca net uptake from 5.38 ± 0.57 ($n = 14$) to 3.85 ± 0.18 ($n = 16$) and 3.39 ± 0.24 ($n = 16$) respectively ($p < 0.01$ in both cases). The ^{45}Ca net uptake obtained with 10^{-3} M gentamycin was very close (63% of the control) of the Ca^{2+} threshold whereas the amount of Ca incorporated in the presence of 10^{-4} M gentamycin reached supra-threshold values (71% of the control), sufficient to release twice the amount of insulin when compared to the basal value obtained in the absence of gentamycin and glucose. The inhibition of insulin release and the reduction of the ^{45}Ca net uptake induced by gentamycin is very similar to that we obtained recently using sisomycin, an aminoglycoside antibiotic of the gentamycin family[14]. The observation that increasing extracellular Ca^{2+} concentration abolished or at least significantly reduced the effect of sisomycin is in good agreement with the idea that gentamycin exerts its inhibitory effect on the insulin release mainly by lowering the uptake of Ca^{2+} by the islets. In the absence of Ca^{2+}, with or without EGTA, Ba^{2+} is the unique agent able to activate the process of insulin release[15,16]. This process can be potentiated by theophylline[16,17] and it is assumed to result from a direct activation of the effector system responsible for the extrusion of insulin-containing granules, namely the microtubules-microfilaments and membranes. Indeed Ba^{2+} plus theophylline (2 mM each) significantly stimulated insulin release from 33 ± 1.6 ($n = 14$) to 113 ± 12.3 ($n = 13$) μU/islet per 90 min ($p < 0.01$). In order to investigate possible effects of gentamycin on the effector system we tested 10^{-4} M gentamycin (which significantly reduced both insulin release and ^{45}Ca net uptake) on the insulin secretion induced by Ba^{2+} and theophylline. Under these conditions the insulin release averaged 114 ± 11.4 ($n = 14$) μU/islet per 90 min, a value not statistically different ($p > 0.95$) from the above control. In conclusion, these results suggest that gentamycin may inhibit insulin release by blocking the entry of Ca^{2+} into the B-cells, instead of having a deleterious effect on a more distant event in the secretory sequence, i.e., the extrusion of the B-granules.

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Studies on the fissure of cholesterol-pigment-calcium stone (multiple faceted stone)

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Summary. Multiple faceted gallstones with an internal fissure have been examined by gas chromatography-mass spectrometry. In vivo, the fissures probably contain only water vapor. The fissure may be produced by dehydration or water trapping during the stone formation process.

It is well known that many cholesterol-pigment-calcium stones (multiple faceted stones) possess a characteristic fissure[1-3] in the central part as shown in figure 1. However, little attention has yet been focused on the contents or causation of the fissures. In the present paper, the authors report some analytical results on the contents of the fissures of multiple faceted stones using gas chromatography-mass spectrometry (GC-MS), and discuss the formation process of multiple faceted stones with fissures in comparison with non-fissured stones.

Materials and methods. 14 multiple faceted stones, obtained from gall bladders which were not inflamed but had slightly infected bile, were studied. The average content of cholesterol in the stones was $80 \pm 12\%$ by weight. 12 stones kept under room conditions for severeral months were classified on the basis of their soft X-ray findings[4] into 2 groups: 8 stones (group A) had fissures and 4 stones (group B) did not. 2 fresh stones were placed immediately after operation in an isotonic physiological saline solution (group C).

GC/selected ion monitoring MS: A JEOL-JMS-D100 GC-MS was used for analyzing the content of the fissures. The operating conditions were as follows: ionization energy, 75 eV; ion source temperature, 200 °C; accelerating voltage, 3 kV; ionizing current, 300 μA; ion multiplier, 1.5 for m/z 28, m/z 32 and 0.3 for m/z 18. A stainless steel column (1.5 ml × 3 mm i.d.) packed with Carbosieve S100/200 mesh (Supelco, Inc.) was used, and the column temperature was held at 60 °C for 8 min and programmed to 120 °C at a rate of 15 °C/min. Each stone was placed in a teflon tube (10 cm 1 × 10 mm i.d.) for a few minutes in order to exclude air and water around the stone. GC-MS was started immediately after crushing the stone by pressing the tube. Under these conditions the calibration curves were obtained by plotting the ratio of peak areas against the injected amounts.