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Inhibition of Pancreatic Secretion by the Carbonic Anhydrase Inhibitor, 2-Acetylamino-1,3,4-Thiadiazole-5-Sulfonamide (Compound No. 6063).* (19767)

DAVID BIRNBAUM† AND FRANKLIN HOLLANDER.

From the Gastroenterology Research Laboratory, The Mount Sinai Hospital, New York City.

Carbonic anhydrase, the enzyme concerned with the hydration of CO_2 and, consequently, the formation of bicarbonate, occurs in significant concentration in the pancreas(1). Tucker and Ball(2) attempted to demonstrate that carbonic anhydrase plays a major role in the formation of bicarbonate by the pancreas. For this purpose, sulfanilamide, an inhibitor of the enzyme *in vitro*, was injected into anesthetized dogs with cannulated pancreatic ducts secreting in response to secretin. Failure of these efforts led the investigators to conclude that this enzyme plays no essential role in pancreatic secretion. Recently, a new carbonic anhydrase inhibitor (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, designated compound No. 6063) which may be as much as 440 times more potent than sulfanilamide *in vitro*, has become available(3,4). This agent has already been found to reduce the secretion of histamine-induced gastric HCl considerably, when administered intravenously to Heidenhain pouch dogs(5), or when applied to the mucosa topically(6). Hence, we undertook to study the effect of this drug on pancreatic secretion, and the results are reported herewith.

Methods and materials. Observations were made on 3 mongrel dogs, provided with duodenal fistulae for collection of pancreatic juice according to Thomas(7). Experiments were never done less than 4 weeks after operation, nor at intervals of less than one week. The dogs were fasted 24 hours prior to each experiment. Following cannulation of the main pancreatic duct, unstimulated secretion was collected for one hour. Then Secretin, Lilly (5 units/kg) was injected intravenously and the secretory response was followed for one hour (control period, A). Immediately after this second hour, the inhibitor, dissolved in a minimum of NaOH solution, was injected slowly intravenously. Two or 3 hours after the inhibitor had been administered, a second injection of secretin was given and the secretion collected for the following hour (period B). The same procedure was repeated 5 hours after injection of the inhibitor (period C). Pancreatic juice specimens were collected for successive 10-minute intervals and all volumes were recorded; those containing less than one ml were pooled consecutively until a specimen with at least this volume was obtained. The concentration of total CO_2 (*i.e.*, dissolved gas plus bicarbonate) in each sample was determined in duplicate, using the constant volume method of Van Slyke(8). All determinations were performed with as little delay as possible —never more than 5 hours after collection. In all, 18 experiments were performed, using compound No. 6063 in dosages of 2, 5, 10, 30, 40 or 60 mg/kg. There were no general toxic signs following the slow intravenous injection of the drug, in conformity with previous observations(9) in this laboratory.

The extent of inhibition was measured in terms of volume of secretion and bicarbonate concentration, by comparison of the *hourly* response to secretin for period A (without inhibitor) with the responses for periods B and C. Percentage inhibition for each experiment was calculated in terms of the decrease

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† Warburg-Magnus Fellow from the Hadassah-Hebrew University Hospital, Jerusalem, Israel.

in hourly response relative to the corresponding control period.

Results. There was clear evidence of a reduction in volume response to secretin following the intravenous administration of compound No. 6063 in all experiments, except one at the lowest dosage level. Of the remaining 17 experiments, the degree of inhibition was less than 20% in 1, 20-50% in 7, 51-70% in 3, and 71-96% in 6. It is noteworthy that 13 of these 17 experiments show a greater degree of inhibition in period B than in period C.

Bicarbonate concentration was also reduced in most of the experiments, the percentage inhibition being distributed as follows: 0-10% in 2 experiments, 11-20% in 5, 21-40% in 7, and 41-60% in 4 experiments, but none over 60%. In period A, with secretin alone, the concentration of bicarbonate varied directly with the volume-rate of secretion, in conformity with previously published studies. In periods B and C, following administration of the carbonic anhydrase inhibitor, the bicarbonate concentration was generally lower than would be expected from the relation in period A. Hence, it appears that the reduction in bicarbonate concentration effected by the inhibitor cannot be attributed solely to the corresponding decrease in volume, but is greater than would be expected solely from the control concentration-volume correlation.

For both volume and bicarbonate concentration, the magnitude of the inhibition appeared to be unrelated to the dosage of compound No. 6063 in the range studied.

Comment. The results of this study demonstrate the influence of a drug, which is a potent carbonic anhydrase inhibitor *in vitro*, on pancreatic secretion *in vivo*. It is conceivable that the reduction in secretion resulted only indirectly, that is, from the influence of compound No. 6063 on some other bodily function which in turn affected pancreatic activity, but our experience with this inhibitor in gastric secretion suggests that this is unlikely. The highest degree of volume inhibition attained was 95%; it never reached 100%. The residual 5% may possibly be explained in part as representing the secretion of bicarbonate formed from the reaction $CO_2 + H_2O \rightarrow H_2CO_3$ in the absence of catalysis. Another important consideration is the concentration of the drug within the pancreatic cell, which may not have sufficed to inhibit its entire content of carbonic anhydrase.

Summary. Intravenous injection of 2-acetylamino - 1,3,4 - thiadiazole-5-sulfonamide (compound No. 6063) in pancreatic fistula dogs results in a marked reduction in the volume output and the bicarbonate concentration of pancreatic secretion following stimulation with secretin. Since this compound is a potent inhibitor of carbonic anhydrase activity *in vivo*, it is likely that this reduction in secretory activity results from a similar inhibition of the carbonic anhydrase present in the pancreas. This supports the view that this enzyme plays a dominant role in bicarbonate formation by this gland.

Statistical analyses of the concentration-volume data will be published later in a detailed report of this work.

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