Insulin Response in Pancreatectomized Dogs Treated with Oxytetracycline

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SUMMARY

Treatment with oxytetracycline seems to prolong and intensify the activity of insulin administered to pancreatectomized dogs. Oxytetracycline-treated dogs can be kept normoglycemic with small doses of insulin, have nondiabetic glucose tolerance, and exhibit the impaired hyperglycemic and cardiac responses to epinephrine of normal animals treated with insulin. DIABETES *19*:307-11, May, 1970.

In a previous investigation[1] we observed that the administration of oxytetracycline to pancreatectomized dogs maintained with intravenous fluid and insulin resulted in marked hypoglycemia with convulsions unresponsive to glucose administration. These hypoglycemic animals had normal or moderately elevated liver glycogen, nondiabetic glucose tolerance, and virtually no hyperglycemic response following epinephrine injection. Owing to the profound hypoglycemia, insulin sensitivity was not tested. The present investigation was designed to observe the effect of oxytetracycline in insulin-treated diabetic dogs *before* the pancreatectomized animals developed marked hypoglycemia and intractable convulsions.

METHOD

Twelve mongrel dogs of either sex, weighing between 10 and 16 kg., were used (seven experimental animals and five controls). After a twenty-four-hour fast they were anesthetized by the intravenous injection of sodium pentobarbital (25 mg./kg.) and pancreatectomized by the avulsion technic.[2] After the operation, a polyethylene catheter was fixed in an external jugular vein and subsequently used for the continuous infusion of maintenance fluid. In experimental dogs, the maintenance fluid was 75 ml./kg./24 hrs. of a solution of 5 per cent glucose in 0.075 M NaCl that contained in each liter 15 U. of insulin (Regular Iletin), 20 mEq. KCl, 20 mg. methionine, 200 mg. choline, 2 ml. water-soluble vitamins (Solu-B) and 250 mg. of oxytetracycline (Terramycin). Blood glucose was determined each morning by a rapid enzyme strip method (Dextrostix). When the morning blood glucose fell to about 130 mg./100 ml., the insulin added to each liter of fluid was reduced to 10 U.; when the glucose level again fell to between 90-130 mg./100 ml., the added insulin was reduced to 5 U. per liter. When the morning blood glucose subsequently fell to around 90 mg./100 ml., the animals' glucose tolerance was tested and, following this, the responses to epinephrine and sensitivity to insulin were determined (figure 1). Following the last test, three dogs were anesthetized and specimens of liver obtained for glycogen determination.

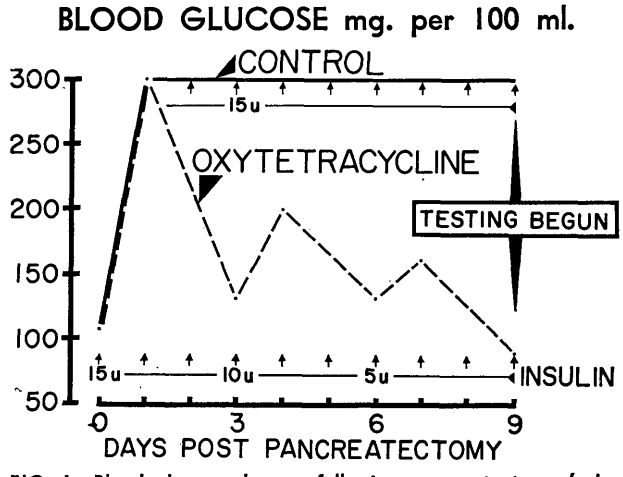

FIG. 1. Blood glucose changes following pancreatectomy (schematic).

The five control animals were treated in the same way as the experimental ones except that the intravenous maintenance fluid contained no oxytetracycline, and its insulin content was kept at 15 U. per liter throughout the experiment (figure 1).

Two hours before each test, the insulin infusions were discontinued. In experimental dogs, the maintenance fluid was replaced with 0.15 M NaCl containing 250 mg. oxytetracycline per liter; in controls, with 0.15 M NaCl alone. Two hours is a period normally long enough for insulin activity to become negligible.[3] Before the insulin sensitivity test in an experimental animal enough 10 per cent glucose solution was admin-

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istered to raise the blood glucose to around 200 mg./100 ml.; this was done in order to avoid excessively long periods of hypoglycemia and convulsions.

Glucose tolerance was determined by the intravenous injection of 0.6 gm. of glucose/kg., the response to epinephrine by the intravenous injection of 25 μg./kg. of epinephrine (Adrenalin Chloride), and insulin sensitivity by the intravenous injection of 0.25 U. of insulin/kg. Venous blood specimens were collected at appropriate intervals for one-and-a-half to eight hours (figures 2, 3, 4). Tests were generally done on successive days. We found that a steady state could be most nearly maintained if, after each test, the infusion of maintenance fluid with insulin was resumed at a rate that administered 75 ml./kg./24 hrs. by the following morning. Control dogs were treated in the same way and tested on the same days after pancreatectomy as were the experimental animals (see below).

Serum bilirubin, SGOT and BSP were determined in several control and experimental animals. Glucose was determined by a glucose-oxidase method;[4] liver glycogen was extracted by the method of Good et al.[5] and quantitated by the anthrone method.[6]

RESULTS

In control dogs, the mean morning glucose level was usually over 250 mg./100 ml., except some mornings after testing was begun, when the accelerated infusion of maintenance fluid resulted in a lowering of the blood glucose. Thus the mean preinjection level before the glucose tolerance test was 301 mg./100 ml. (195 to 407); before determination of the response to epinephrine, 156 mg./100 ml. (91 to 306); and before testing of insulin sensitivity, 243 mg./100 ml. (146 to 340). The results of the tests were typical for those of diabetic animals. The blood glucose rose steeply in the glucose tolerance test, with no return to the preinjection level (figure 2). The response to epinephrine was sharp, with a continuing rise of blood glucose, so that at the end of ninety minutes the mean value was 185 per cent greater than the average preinjection level (figure 3). Other effects of epinephrine, such as tachypnea and tachycardia, were also present. The response to the test dose of insulin was a mean fall in blood glucose of 68 per cent (to about 80 mg./100 ml.) within one hour. After ninety minutes the glucose began to rise, and at the end of five hours it was about 80 per cent of the initial value—about 200 mg./100 ml. (figure 4).

In experimental animals treated with oxytetracycline and diminishing amounts of insulin, the morning blood glucose levels eventually became nearly normal or even

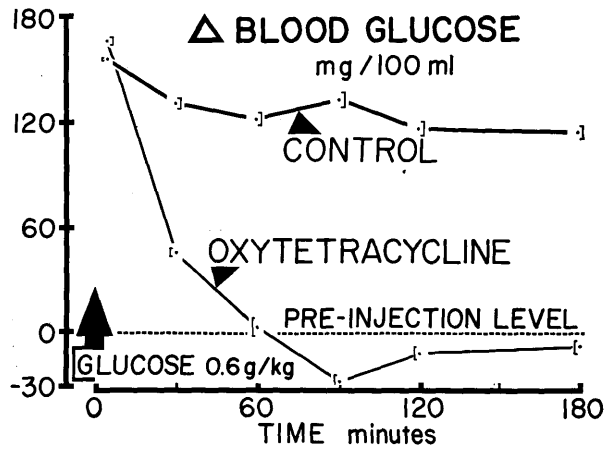

FIG. 2. Glucose tolerance of pancreatectomized dogs. Vertical lines represent S.E.M. (omitted where less than 2).

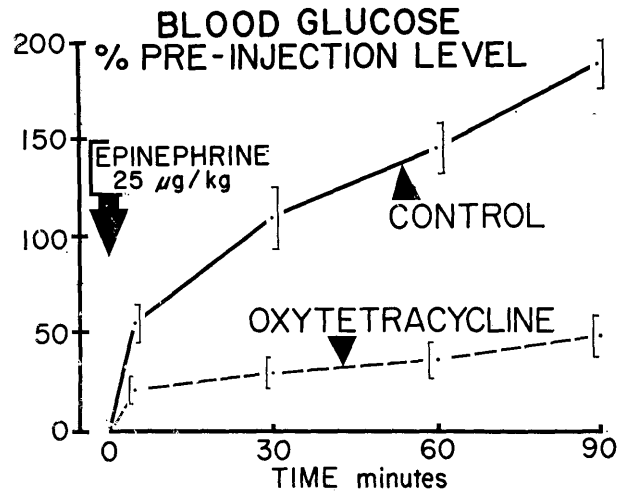

FIG. 3. Response to epinephrine in pancreatectomized dogs.

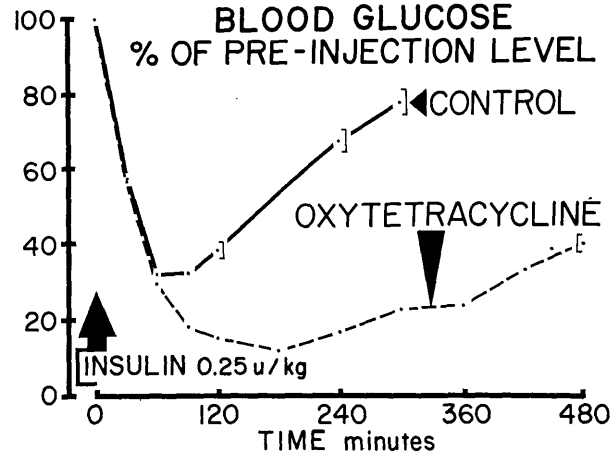

FIG. 4. Insulin sensitivity of pancreatectomized dogs.

subnormal, but the length of treatment needed to attain this state varied widely—between three and thirteen days (mean = nine days). The mean level before the glucose tolerance test was 111 mg./100 ml. (57 to 146); before determination of the response to epinephrine, 90 mg./100 ml. (48 to 130). Before testing insulin sensitivity, the mean level of 102 mg./100 ml. (54 to 126) was raised by the infusion of 10 per cent glucose to 214 mg./100 ml. (156 to 250).

The responses of the experimental animals to the three tests were quite different from those of the controls. The glucose tolerance was nondiabetic; within sixty minutes blood glucose returned to the normal preinjection level and subsequently fell below it (figure 2). The response to epinephrine was sharp but there was only moderate blood glucose elevation which failed to rise significantly; at the end of ninety minutes the mean increase in blood glucose was 47 per cent, about one fourth that observed in the controls (figure 3). Other effects of epinephrine such as tachypnea and tachycardia were not pronounced or absent. The response to the test dose of insulin was marked; a 70 per cent fall in the level of blood glucose within one hour, a maximum fall of 88 per cent at the end of three hours (when the level was around 30 mg./100 ml.), and a subsequent rise that was very gradual, so that at the end of eight hours the glucose value was still only 40 per cent of the preinjection level—about 90 mg./100 ml. (figure 4). The liver glycogen of the dogs tested averaged 72 mg./gm. (64 to 78), well within the usual range we find in the pancreatectomized dogs in our laboratory. None of the animals tested in this or the previously reported experiments[1] showed evidence of hepatic dysfunction.

DISCUSSION

In the experiments previously reported,[1] the pancreatectomized dogs were maintained on a continuous infusion of a solution containing 250 mg. oxytetracycline and 15 U. of insulin. On this regimen they developed marked hypoglycemia and convulsions that did not subside with intravenous injections of glucose. In the present experiment, by monitoring blood glucose daily and reducing insulin dosage as indicated, convulsions were avoided and tests carried out on apparently normal animals that were, at most, only moderately hypoglycemic.

The results of the present experiment suggest that oxytetracycline may intensify and prolong the activity of insulin in pancreatectomized animals. After intravenous injection of a test dose of insulin, the blood glucose response of control and experimental dogs was the same during the first hour only. Thereafter in the experimental group the blood glucose continued to fall for two more hours, to 12 per cent of the preinjection level, at which time a very gradual rise began. After eight hours the blood glucose level was still only 40 per cent of the preinjection value (figure 4). It should be noted that in these animals the preinjection level was not the true one. The first two insulin tolerance tests ended after two hours with blood glucose at undetectable levels and the dogs convulsing; the others were done in animals primed with 10 per cent glucose. Blood glucose was approximately doubled by the priming injection, thus adding the effect of acute glucose load to the insulin sensitivity test. But in experimental dogs the effect of acute glucose load lasted for only about an hour, i.e., within that time the blood level returns to the preinjection value (figure 2). In experimental dogs loaded (primed) with glucose and given 0.25 U./kg. of insulin, the blood glucose at the end of an hour was almost the same as in the controls (figure 4), suggesting that in the presence of a fairly large dose of insulin the effect of glucose load was insignificant.

Persistence and intensity of the insulin effect could explain why a pancreatectomized dog treated with oxytetracycline has a normal glucose tolerance curve two hours after the administration of insulin is discontinued, when the activity of the hormone should be negligible. It also could explain why such a dog maintained by the intravenous infusion of fluid containing only 5 U. of insulin for each 50 gm. of glucose has a normal or even subnormal blood glucose (figure 1) and ample liver glycogen. Oxytetracycline alone has no hypoglycemic effect, for when the small amount of insulin in the maintenance fluid of experimental dogs is omitted, the usual hyperglycemia of diabetes recurs within twenty-four hours. Furthermore, the negative liver function test, the microscopic appearance of the organ[1] and the ready mobilization of its glycogen by glucagon[1] make it unlikely that oxytetracycline acts by way of a toxic hepatic effect that depresses glucose formation.

Insulin and epinephrine have antagonistic effects on blood glucose, and in both animals and man insulin hypoglycemia can elicit a secretion of epinephrine that acts to elevate the blood glucose.[7] In the control dogs, the source of pancreatic glucagon had been removed, and intestinal glucagon presumably was not released in the absence of alimentation.[8] In the controls, endogenous release of epinephrine probably contributed significantly to the rise of blood glucose that follows the hypoglycemia induced by a test dose of insulin, for

there was prompt response following exogenous epinephrine (figure 3). In oxytetracycline-treated dogs, the response to epinephrine was impaired. Despite the presence of ample liver glycogen, a large test dose elicited only a small rise of blood glucose (figure 3). In such animals, even if insulin hypoglycemia should stimulate a normal response of the adrenal medulla, the epinephrine released would probably not raise blood glucose significantly; the impaired hyperglycemic response to epinephrine would manifest itself as a prolonged and intensified hypoglycemic response to the test dose of insulin.

It is possible also that oxytetracycline enhanced the activity of insulin by interfering with that of epinephrine, or that oxytetracycline increased the half-life of insulin. Normal dogs treated with 0.25 U./kg. of insulin have no hyperglycemic or positive chronotropic response to an injection of 25 μg./kg. of epinephrine administered seven minutes later; their response is essentially that of the experimental animals described in this communication.[9]

The tetracyclines are known to influence carbohydrate metabolism and insulin. Experimentally, Prochazka et al.[10] found that the hormone is bound by chlortetracycline, although biological activity of the complex was not determined. Koshik and Grishillo[11] reported that tetracycline administration raised the liver glycogen of white mice. Banerjee et al.[12] observed that tetracycline, when administered to monkeys and guinea pigs, raised the fasting blood glucose and diminished glucose tolerance. We have observed that intraperitoneal injection of oxytetracycline into intact white rats is followed by at least a doubling of the blood glucose and an increase of the liver glycogen of about 25 per cent. On the other hand, we have also observed that the intravenous administration of oxytetracycline alone does not affect the blood glucose levels of normal dogs nor the hyperglycemia of diabetic ones.

DeLollis and Privitera[13] reported that administration of oxytetracycline to healthy diabetic humans lowers the fasting blood glucose and improves the glucose tolerance. Miller[14] also reported two cases of change in glucose tolerance after treatment with oxytetracycline. One, a young diabetic with infectious mononucleosis who needed fairly large doses of insulin for control, became hypoglycemic when oxytetracycline was administered. The hypoglycemia abated when the drug was discontinued, only to reappear when the antibiotic was resumed. In a case that we observed, oxytetracycline was used to treat a foot infection in an elderly, long-standing diabetic who had been well controlled with moderate amounts of tolbutamide (Orinase). The patient developed hypoglycemia and a nondiabetic glucose tolerance that persisted after tolbutamide was discontinued. The hypoglycemia ended only after oxytetracycline was discontinued. Healthy skeletal muscle obtained at amputation contained 1.6 per cent glycogen, well above normal. The patient's age was too advanced and his condition too precarious to permit further studies.

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