

## SUMMARY

Treatment of male rats with alloxan (120 mg/kg, S.C.), 6-aminonicotinamide (35 mg/kg, I.P.), N-methylacetamide (6.25 ml/kg, P.O.) and streptozotocin (55 mg/kg, I.P.) all produced hyperglycemia which was both time and dose dependent. The hyperglycemia reached its plateau at 24 hours after 6-aminonicotinamide, N-methylacetamide and streptozotocin. However, it was maximum at 72 hours after alloxan. N-methylacetamide but not other agents caused a small but significant increase in SGPT level. This pretreatment significantly prolonged hexobarbital sleeping time without affecting the awakening brain barbiturate level. Pharmacokinetic studies of hexobarbital, aminopyrine, phenylbutazone and sulfapyridine in these diabetic animals also showed an increase in the plasma half-life of the drug without any change in its volume of distribution. However, the half-life of aniline was increased in the rats treated with 6-aminonicotinamide, N-methylacetamide and streptozotocin. It was decreased (about 40 %) in alloxan-treated animals.

These four chemicals were also found to depress hepatic microsomal aminopyrine N-demethylase activity even though hepatic microsomal protein and cytochrome P-450 content were unaffected. In fact, aniline hydroxylase activity was increased by about 30 % in animals treated with this dosage of alloxan. Moreover, the time course of streptozotocin treatment on aniline hydroxylase activity was found to be biphasic : it was decreased in the first and second days but later on showed an increase.

Characterization of aminopyrine N-demethylase by kinetic studies revealed that the enzymes from control and chemical diabetic (alloxan, 6-aminonicotinamide and streptozotocin) were similar; however, a significant increase in  $K_m$  value was found in the N-methylacetamide-treated group a qualitative change in this enzyme. This evidence was further supported by both the inhibition studies using SKF-525 A and p-chloromercuribenzoate and heat denaturation studies. Similar kinetic studies on aniline hydroxylase of the microsomes derived from animals treated with 6-aminonicotinamide, N-methylacetamide and streptozotocin showed a similar quantitative property whereas the enzyme from alloxan treated animals revealed both quantitative and qualitative alterations. Glucose, insulin and all four diabetogens had no direct effect on the drug-metabolizing enzymes in vitro.

Electron microscopic studies showed that the hepatocytes from rats treated with alloxan and streptozotocin were altered; depleted hepatic glycogen, dilated endoplasmic reticulum and abundant lipid droplets are clearly discerned. The parallel pattern of endoplasmic reticulum normally seen in control hepatocyte disappeared after 6-aminonicotinamide treatment. However, some degrees of cell damage was observed in liver cells from N-methylacetamide treated rats.

The changes (increase or decrease) in hepatic drug metabolism in these diabetic animals were found concurrently with the depletion of hepatic glycogen and a reduction in the circulating level of testosterone.

However, this reduction in plasma testosterone level was not likely to be the cause responsible for the decrease in hepatic drug metabolism, since testosterone supplement (12 mg/kg, S.C., once daily for 2 days) could not restore the normal aminopyrine N-demethylase activity. Moreover, no correlation could be demonstrated between the activities of microsomal drug-metabolizing enzymes and the hepatic glycogen content.

Combination studies using microsomes and supernatant fractions from control and treated animals were consistent with the view that the focus of the effects of these diabetogens on hepatic drug metabolism was not cytosolic but was most likely associated with a qualitative or quantitative change in the enzyme from the microsomal fraction itself. This possibility was also supported by the fact that sodium dodecyl sulfate/polyacrylamide gel-electrophoresis protein profiles exhibited by diabetic microsomes were different from the one shown by normal microsomes.

In all cases, however, insulin (20 units/kg, S.C., once daily for 2 days) could antagonize alloxan-and streptozotocin- induced changes in liver and body weight, blood glucose and activities of these drug-metabolizing enzymes. Pharmacokinetic studies of hexobarbital, aminopyrine, phenylbutazone, sulfapyridine and aniline in alloxan- or streptozotocin-diabetic rats given insulin supplement also showed pharmacokinetic profiles similar to those of the control.

Experimental diabetes also affected the extent of drug binding to the plasma proteins. It was found that there was a significant reduction

in plasma protein concentration in all diabetic groups except that with 6-aminonicotinamide. Hypertriglyceridemia was also found in every diabetic group. Plasma protein binding of phenylbutazone or sulfapyridine was significantly reduced, especially at high drug concentrations when investigated by using the plasma from rats treated with alloxan, N-methylacetamide and streptozotocin, while the binding of acetylsalicylic acid showed a small decrease only with plasma protein from rats treated with N-methylacetamide and streptozotocin. Insulin supplement to alloxan- and streptozotocin-diabetic rats could also restore the normal binding of the drugs. Moreover, it was found that experimental diabetes caused by 6-aminonicotinamide caused a significant reduction in hepatic blood flow.

The present studies have clearly shown that experimental diabetes could affect drug disposition in vivo by altering in drug protein binding, hepatic blood flow and hepatic drug metabolism. The possible mechanisms responsible for these alterations have been discussed. All abnormalities that were noted in the diabetic state caused by alloxan or streptozotocin treatment were antagonized by insulin supplement. These experimental animals may constitute a good model for further studies regarding drug dosage and dosing interval in patients treated or untreated with insulin or other antidiabetic agents.

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