Treatment of Streptozotocin Diabetes with Di-isopropylammonium Dichloroacetate (DIPA)

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SUMMARY

Streptozotocin-diabetic rats were treated with di-isopropylammonium dichloroacetate (DIPA) via an orogastric tube in doses of 25 or 50 mg./1 kg. twice daily for one week. In nonketotic animals, mean blood glucose concentration decreased significantly during treatment with either DIPA or its acid moiety, sodium dichloroacetate. Neither di-isopropylammonium hydrochloride nor saline reduced hyperglycemia in the diabetic rats. There was no change in the blood glucose of nondiabetic rats in response to DIPA. In ketotic diabetes produced by streptozotocin, 63 per cent of DIPA-treated animals survived after one week, compared to 24 per cent of those given saline. DIPA did not influence glucose absorption in isolated everted jejunal sacs from diabetic and normal rats, while phenformin inhibited transport by more than 50 per cent. Thus DIPA, which only partially mimics insulin in its actions and does not stimulate insulin release as do the sulfonylureas, also fails to duplicate the effects of phenformin and appears to have a unique mode of action. DIPA is an effective oral hypoglycemic agent in streptozotocin-diabetic rats. DIABETES *23*:179-82, March, 1974.

In 1962 Lorini and Ciman discovered that di-isopropylammonium dichloroacetate (DIPA), a vasodilator, lessened hyperglycemia of alloxan-diabetic rats.[1] When given intraperitoneally in a dose of 400 mg./1 kg. body weight, blood glucose concentrations were reduced significantly, while normal rats were unaffected. This finding was confirmed by Stacpoole and Felts,[2] who also showed that DIPA and sodium dichloroacetate (DCA), the sodium salt of its acid moiety, but not di-isopropylammonium hydrochloride (DIA), stimulated glucose oxidation and inhibited fatty acid oxidation in muscle from diabetic, but not from nondiabetic, rats.

To evaluate the potential effectiveness of DIPA as an oral hypoglycemic agent in diabetes mellitus, the drug was administered via an orogastric tube to streptozotocin-diabetic rats in the present study. Its effect on blood glucose in nonketotic animals and its ability to improve survival in ketotic animals were studied. In addition, DIPA was compared with phenformin as an inhibitor of glucose transport in the small intestine.

MATERIALS AND METHODS

All rats in these experiments were housed individually in metabolic cages with a twelve hour period of light and had free access to standard laboratory chow and drinking water. DIPA (obtained from Delmar Laboratories, Montreal, Canada) was administered as a 10 mg./1 ml. solution in distilled water. DCA (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) and DIA neutralized with hydrochloric acid (Baker Grade, Baker Chemical Co., Phillipsberg, N.J.) were given as 5 mg./1 ml. solutions. Streptozotocin (kindly provided by Dr. George C. Gerritsen, Upjohn Company, Kalamazoo, Mich.) was freshly prepared in citrate buffer at pH 4.5 before injection. Urinary ketones were measured as acetoacetic acid by a nitroprusside reaction (Ketostix, Ames Co., Elkhart, Ind.) and blood glucose by a glucose oxidase test strip method using a reflectance meter (Dextrostix, Ames Co.).

Nonketotic diabetes was produced in 250 gm. male Long-Evans rats by intravenous injection of 65 mg./1 kg. streptozotocin. Daily venous blood glucose values were obtained for three days at the end of the first week following streptozotocin, and the animals were randomly assigned to one of the treatment groups if the mean pretreatment blood glucose was greater than 300 mg./100 ml. Groups of rats were treated with DIPA in doses of 50 or 25 mg./1 kg., DCA, 12.5 mg./1 kg., DIA, 12.5 mg./kg., or normal saline in a similar volume. The agents were administered via an orogastric tube under light ether anesthesia every

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twelve hours during the second week. Blood glucose values were calculated as a mean of three determinations made each evening at the end of the treatment week and again after a third week without medication. Two groups of rats that had not been made diabetic were given either DIPA, 50 mg./1 kg., or saline and their glucose measured in the same manner as described above. The average of pre- and post-treatment gucose values was subtracted from the treatment value to derive the net change, and significance was calculated on this change by paired *t* test.

Male Sprague-Dawley rats weighing 400 gm. were given intravenous injections of streptozotocin, 100 mg./1 kg., to produce ketotic diabetes. Thirty-six animals that had begun to excrete large amounts of urinary acetoacetic acid within twenty-four to seventy-two hours were immediately treated with either DIPA, 25 mg./1 kg., or saline by intragastric instillation every twelve hours. The treatment was maintained for one week in surviving animals, and any change in the urinary ketone reaction was noted. Survival and disappearance of urinary ketosis in the two groups were compared, and significance was determined by chi-square analysis.

The effect of DIPA on intestinal glucose transport was assessed in rat jejunal sacs by the technic of Wilson and Wiseman[3] as modified by Kruger et al.[4] and compared to that of phenformin. Normal or streptozotocin-diabetic male Long-Evans rats, weighing 250 gm., were given a single intragastric dose of DIPA, 50 mg./1 kg., phenformin, 25 mg./1 kg., or saline two hours before they were killed by a blow on the head and the small intestine was removed. Three jejunal sacs, 7 cm. in length, were prepared from each animal by everting the segments and tying the ends. The sacs were filled with 1 ml. of a saline solution containing 0.004 M. tris buffer, 0.0001 M. calcium chloride and 200 mg. /100 ml. glucose at pH 8.5 and placed in separate flasks containing 15 ml. of the same solution. These were then incubated at 37° C. for thirty minutes under 95 per cent oxygen and 5 per cent carbon dioxide in a shaking incubator. The glucose concentrations of the fluid within the sacs (I) and the surrounding medium (E) were then determined by a glucose oxidase method and glucose transport (T) was derived from the interior (serosal) and exterior (mucosal) glucose concentrations by the formula

$$T = \frac{I - E}{E}.$$

Significance of the mean transport in rats treated with DIPA or phenformin compared to saline controls was determined by Student's *t* test.

RESULTS

The blood glucose responses of diabetic rats to 25 or 50 mg./1 kg. DIPA and to saline are compared in figure 1. Glucose levels were markedly lower during therapy with either dose of DIPA and subsequently returned to pretreatment levels, indicating that improvement was not due to a spontaneous remission. There was no significant difference in response to the two doses. The mean blood glucose values during treatment with DIPA, DCA, DIA and saline are presented in table 1. While saline and DIA had no effect on hyperglycemia in diabetic animals, DIPA and DCA caused a significant reduction. In all but one of the DIPA-treated rats and in all of the DCA-treated animals, glucose decreased greatly, from 66 to 249 mg./100 ml. Treatment with 50 mg./1 kg. DIPA for one week produced no significant effect on blood glucose concentrations in healthy, nondiabetic rats.

As depicted in table 2, 37 per cent of the ketotic rats treated with DIPA died during the week of therapy while 76 per cent of saline-treated animals died. There was no evidence of ketosis in over half of the DIPA-treated survivors but only one of four survivors in the saline group was free of urinary acetoacetic acid. With regard to both survival and freedom

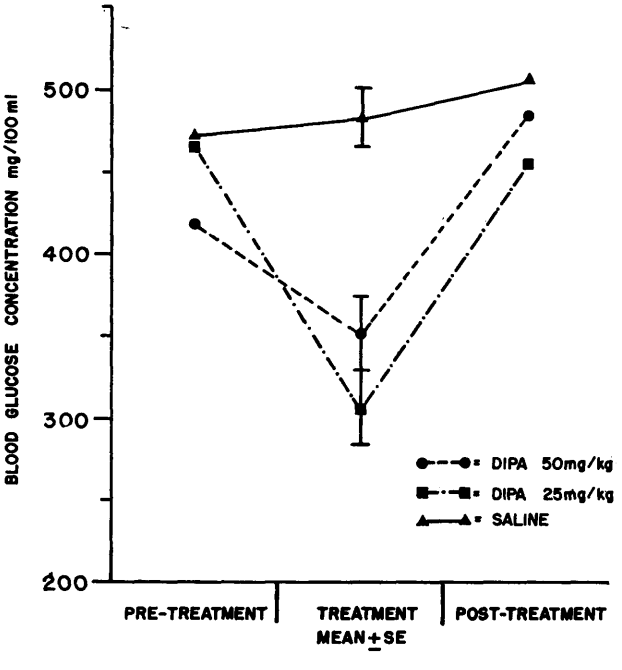

FIG. 1. Comparison of blood glucose responses of streptozotocin-diabetic rats to 25 or 50 mg./1 kg. DIPA or to saline given twice daily in intragastric doses for one week.

TABLE 1

Effect of oral administration of DIPA, DCA or DIA for one week on blood glucose levels in diabetic and nondiabetic rats

	Number	Mean blood glucose (1 mg./100 ml. ± S.E.) Pretreatment	Treatment	Post-treatment	Change*	P
Diabetic						
Saline	8	473 ± 29.1	485 ± 17.1	508 ± 22.5	− 6	N.S.
DIPA 50 mg./1 kg.	10	420 ± 20.9	353 ± 22.9	487 ± 28.7	− 101	<.001
DIPA 25 mg./1 kg.	12	467 ± 34.2	307 ± 23.1	457 ± 33.3	− 155	<.001
DCA 12.5 mg./1 kg.	5	421 ± 51.8	307 ± 48.0	364 ± 53.9	− 85	<.001
DIA 12.5 mg./1 kg.	6	438 ± 41.9	434 ± 41.2	438 ± 23.4	− 4	N.S.
Nondiabetic						
Saline	5	100 ± 1.2	103 ± 4.9	95 ± 3.0	+ 6	N.S.
DIPA 50 mg./1 kg.	5	92 ± 2.7	90 ± 3.2	91 ± 3.1	− 2	N.S.

*Change = $\frac{\text{Pretreatment + post-treatment}}{2}$ − treatment

from ketosis, chi-square analysis showed that DIPA-treated rats were significantly improved compared to saline controls. Blood glucose was not determined in these animals, and urinary glucose reactions did not correlate with the urinary ketones.

Table 3 summarizes the effects of 50 mg./1 kg. DIPA, 25 mg./1 kg. phenformin and saline on glucose transport in jejunal sacs taken from normal and nonketotic diabetic rats. Diabetes more than doubled glucose transport in jejunal sacs from saline-treated rats. Phenformin inhibited glucose absorption by over 50 per cent in both diabetic and nondiabetic rats. DIPA did not affect glucose transport in intestinal sacs obtained from either normal or diabetic animals.

DISCUSSION

The presently available oral hypoglycemic agents have been less than satisfactory as substitutes for insulin. They are not effective in ketosis-prone diabetic patients, and they do not prevent vascular complications of diabetes.[5,6] Moreover, the safety of these drugs has been questioned in recent reports.[5-7] It is therefore necessary to continue the search for oral hypoglycemic agents which have different modes of action than sulfonylureas and biguanides.

Intraperitoneal DIPA was found to significantly diminish hyperglycemia in alloxan-diabetic rats but did not alter glucose levels of nondiabetic animals.[1,2] DIPA acts like insulin in some ways, for example, increasing glucose oxidation to carbon dioxide in diabetic animals[2] and decreasing gluconeogenesis in isolated liver cells from fasted nondiabetic rats.[8] In contrast to insulin it does not increase triglyceride synthesis[2] or glycogen storage.[1] DIPA does not stimulate insulin secretion from the isolated perfused rat pancreas.[9] A proposed mechanism of action for DIPA, based on its inhibition of fatty acid oxidation in diabetic rat muscle[2] and its reduction of elevated citrate concentrations in muscle tissue from diabetic animals,[10] relates the glucose-lowering effect to reactivation of phosphofructokinase, which has been inhibited by elevated intracellular citrate levels secondary to increased fatty acid oxidation. These actions appear to reside in the dichloroacetate component of DIPA.[2]

Since DIPA is absorbed when given orally and is

TABLE 2

Effect of oral administration of DIPA for one week on survival of ketotic diabetic rats

	Number (per cent) Saline	DIPA (25 mg./1 kg.)	P
Ketotic	17	19	
Died of ketosis	13 (76%)	7 (37%)	<.001
Survived	4 (24%)	12 (63%)	<.001
Ketosis cleared	1 (6%)	7 (37%)	<.001

TABLE 3

Effects of DIPA 50 mg./1 kg. and phenformin 25 mg./1 kg. on glucose transport in jejunal sacs from normal and diabetic rats

Treatment	Number	Transport (mean ± S.E.)	P
Normal			
Saline	18	0.36 ± 0.04	–
DIPA	16	0.35 ± 0.04	N.S.
Phenformin	12	0.16 ± 0.03	<.001
Diabetic			
Saline	18	0.75 ± 0.07	–
DIPA	12	0.86 ± 0.13	N.S.
Phenformin	12	0.37 ± 0.04	<.001

relatively nontoxic,[11] its potential use as an oral hypoglycemic agent with a unique mode of action was considered. Both DIPA and its acid moiety, DCA, lessened hyperglycemia during one week of twice daily doses, when compared to the glucose values before and after treatment. The effectiveness of DIPA and DCA, but not DIA, in reducing hyperglycemia is consistent with the in vitro findings of Stacpoole and Felts.[2] DIPA had no effect on the nondiabetic rats in comparison with saline controls, which was also to be expected on the basis of its postulated mechanism of action.

Administration of DIPA, 25 mg./1 kg., twice daily for one week significantly reduces mortality and morbidity of ketotic diabetic rats. Although some ketotic animals seem to survive when untreated, only one of seventeen had a complete, spontaneous remission. In the DIPA-treated group, however, almost two thirds survived and seven of nineteen were free of urinary ketones, apparently a result of therapy. Although neither DIPA nor DCA affect basal fatty acid oxidation in isolated liver cells derived from fasted rats, they tend to reduce hepatic acetoacetate and β-hydroxybutyrate accumulation in the absence of any added gluconeogenic substrate.[8] This action may explain the beneficial effect of DIPA in the treatment of ketotic animals.

Phenformin has some metabolic actions that are similar to DIPA in that it stimulates glucose oxidation[12] and inhibits gluconeogenesis.[13] The primary effect during clinical usage, however, probably results from an impairment in glucose absorption in the small intestine.[4,14] This effect on glucose transport was observed with phenformin but not with DIPA in the jejunal sac experiments. Diabetes, itself, has an effect on glucose absorption, causing an increased uptake of glucose and its analogues.[15] Phenformin inhibits glucose transport in diabetes also, but DIPA has no effect. Thus, DIPA appears not to share with phenformin an action on intestinal glucose transport which is accompanied by bothersome gastrointestinal side-effects in clinical use.

The treatment of experimental diabetes in the rat with DIPA demonstrates that it reduces hyperglycemia and improves survival in ketotic animals but has no effect in euglycemic animals. These actions are in contrast to the sulfonylureas, which stimulate insulin secretion and presumably would not reduce ketosis in animals whose beta cells are severely damaged by streptozotocin. DIPA or similar agents may, therefore, possess significant advantages over the sulfonylureas and biguanides. Further investigation of DIPA or DCA for diabetes treatment appears to be warranted.

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