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HYPOGLYCAEMIC ACTION OF DI*ISO*PROPYLAMMONIUM SALTS IN EXPERIMENTAL DIABETES

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Abstract—The effect of di*iso*propylammonium dichloracetate (DIEDI) on experimental alloxan diabetes has been investigated. After intraperitoneal injection of 0·4 g/kg of DIEDI, the blood glucose and the glycosuria of diabetic rats decrease: the respiratory quotient on the contrary increases, but only during the first 40 min. No significant variation has been noticed after DIEDI treatment on glycogen content of liver and muscle. Addition of DIEDI did not affect glucose uptake by isolated diaphragm of normal and diabetic rats.

DI*iso*PROPYLAMMONIUM salts are used as pharmacological agents for obliterating peripheral vasculopaties[1] and some metabolic disorders due to toxic agents.[2]

However, the mechanism of action of these compounds is still unknown, although Santarato[3] and Santi[4] have pointed out that di*iso*propylammonium salts, and particularly di*iso*propylammonium dichloracetate, affect respiration both *in vivo* and *in vitro* when it is impaired by specific inhibitors, such as tyopentale, KCN and others.

The fortuitous observation that, after administration of di*iso*propylammonium di-chloracetate (DIEDI), diabetic rats underwent a reduction of blood sugar, led us to evaluate the influence of this compound on experimental alloxan diabetes. In particular the action of di*iso*propylammonium dichloracetate on blood glucose, glycosuria, respiratory quotient, liver and muscle glycogen and oxygen uptake by isolated diaphragm of diabetic rats has been studied.

METHODS

Diabetes was reproduced in Wistar CH rats weighing 130–150 g by intravenous injection of 50 mg/kg of alloxan, after 24 hr starvation. Only rats with glycaemia higher than 300 mg/100 ml and persisting for at least 5 days have been used in the experiments.

Blood glucose was determined by the Nelson Somogyi method,[5] urine glucose by the Bertrand method.[6] Respiratory quotients (R.Q.) was determined on samples of dried air, collected from a glass bell (6 l. capacity), in which the animals were kept for 30 min; CO_2 was measured according to Haldane–Margaria[7] and O_2 by means of the Beckman oxymeter using Gaffuri's[8] method. Glycogen was determined by the method of Bomskow and von Kaulla[9].

The glucose uptake by the isolated diaphragm of rat was measured with the technique described by Gemill[10, 11] under the following conditions. Rats fasted for 18 hr were killed by decapitation, and the diaphragm quickly removed and immediately dipped in ice cold Stadie and Zapp solution.[12] Deprived of the central

tendon, the diaphragm was then divided in two or more parts (30–40 mg each) which were carefully blotted with filter paper and weighed on a torsion balance. Each piece was transferred to the side arm of a Warburg flask, previously chilled to 0 °C, containing 0·35 ml of Stadie and Zapp medium in which increasing concentrations (from 15 to 1400 μg) of DIEDI were added. After equilibration for 10 min at 0 °C to obtain equilibrium with the endogenous glucose (see Bornstein and Park[13]), the flasks equilibrated with O_2 were incubated for 120 min at 37 °C. For the analysis of residual glucose, aliquots of medium, before and after incubation, were deproteinized with 5·6% $Ba(OH)_3$ and 5% $ZnSO_4$, and the glucose determined by the Nelson–Somogyi method.[5]

RESULTS

As Fig. 1 shows, administration of DIEDI to alloxan diabetic rats causes a significant lowering of blood glucose (about 60 per cent of the initial value). The effect appears

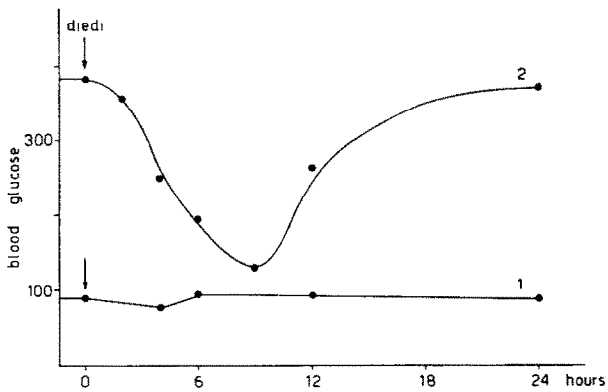

FIG. 1. Blood sugar curves of normal (1) and diabetic (2) rats, fed *ad libitum*, after injection of 0·4 g/kg of DIEDI. Average values from ten animals. The blood glucose is measured in mg/100 ml.

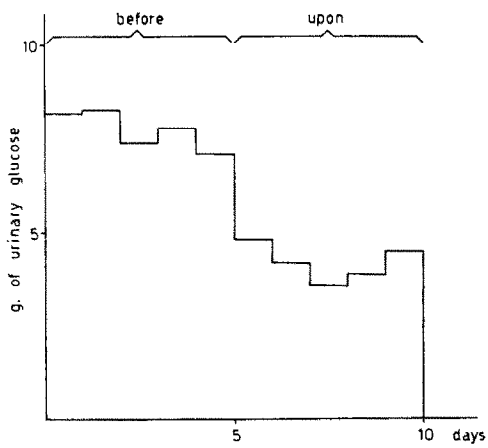

FIG. 2. Urine glucose elimination by diabetic rats fed *ad libitum*, before and upon daily treatment with 0·2 g/kg of DIEDI. Average values from eight animals.

TABLE 1. GLUCOSE UPTAKE BY ISOLATED DIAPHRAGM
(mg/g of fresh tissue, during 120 min)

No.	Normal rats					Diabetic rats					
	Without	Upon addition of DIEDI				Without	Upon addition of DIEDI				
	—	15 μg	70 μg	500 μg	1400 μg	—	15 μg	70 μg	500 μg	1400 μg	Blood sugar (mg/100 ml)
1	4·4	4·4	4·6	—	—	3·3	3·3	—	—	—	580
2	5·9	5·8	5·0	—	—	2·5	2·8	3·8	—	—	570
3	4·3	3·9	4·4	—	—	5·6	5·5	5·0	—	—	300
4	4·9	4·2	4·2	—	—	4·6	4·6	4·0	—	—	350
5	4·8	4·5	4·5	3·7	—	5·1	—	5·5	—	—	320
6	5·8	—	5·8	—	—	4·8	—	4·8	4·9	—	330
7	6·2	—	5·9	—	—	3·9	—	3·2	4·0	—	300
8	5·5	—	—	5·0	4·9	3·8	—	—	4·3	4·2	405
9	5·2	—	—	5·0	5·1	4·1	—	—	4·2	4·6	340
10	4·3	—	—	3·9	3·1	3·5	—	—	—	3·6	380
11	6·5	—	—	6·3	—	3·9	—	—	—	3·7	410
12	3·6	—	—	—	3·8						
13	6·1	—	—	—	5·7						
average	5·2	4·5	4·9	4·7	4·5	4·1	4·0	4·3	4·3	4·0	

2 hr after the injection and reaches the maximum after 8–9 hr. Twelve hours after the injection the glycaemia increases towards the initial values, which are reached within 24 hr. Such an hypoglycaemic action of DIEDI is constant in diabetic animals: no significant variation of blood glucose was observed in normal rats.

The glucose elimination of diabetic rats decreases remarkably after one dose of 0·4 g/kg of DIEDI: this diminution does not persist longer than 24 hr. Daily injection of 0·2 g/kg of DIEDI results in a stable reduction of the glycosuria of about 40 per cent (Fig. 2).

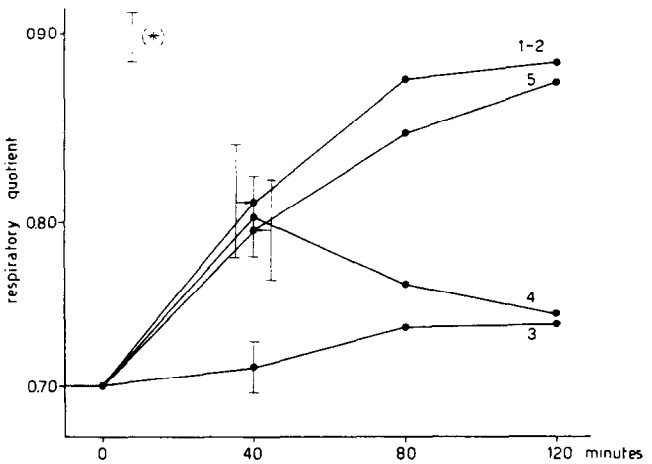

FIG. 3. Respiratory quotient relative to: (1) untreated normal rats; (2) normal rats treated with 0·4 g/kg of DIEDI; (3) untreated diabetic rats; (4) diabetic rats treated with 0·4 g/kg of DIEDI; (5) diabetic rats treated with 20 IU/kg of insulin. All the animals, starved, were injected with 0·4 g/kg of DIEDI 4 hr before the experiment. Glucose (5 g/kg) was injected intraperitoneally at time 0. (*) Mean ± s.e.p.[15]

The action of DIEDI on the R.Q. is visualized in Fig. 3. While the R.Q. in normal animal upon administration of glucose undergoes a remarkable increase, in diabetic animal such an increase is very small or absent.[14] However, if the administration of glucose is preceded by an injection of 0·4 g/kg of DIEDI, the R.Q. of diabetic animals undergoes a significant increase during the first 40 min. The same treatment does not affect R.Q. of normal rats.

The results concerning glucose uptake by isolated diaphragm are shown in Table 1. No significant variation was observed on adding DIEDI (from 15 to 1400 μg for sample) both to normal and diabetic diaphragm.

The results concerning glycogen content of liver and skeletal muscle of normal and diabetic rats are reported in Table 2. Glycogen content of liver and muscle of normal rats is constantly higher than that of diabetic animals. DIEDI treatment does not significantly affect glycogen of liver and muscle of diabetic animals.

DIEDI exhibits a constant hypoglycaemic action in alloxan diabetes, but in contrast with the known hypoglycaemic factors, it does not affect blood glucose of normal animals. The hypoglycaemic action in alloxan diabetes cannot be attributed to an increased urinary elimination of glucose, glycosuria of diabetic rats being constantly decreased (40 per cent) upon treatment with DIEDI. Neither can the hypoglycaemic

action be attributed to an increased synthesis of glycogen, since glycogen content of liver and muscle of diabetic rats does not appreciably change after DIEDI treatment.

The increase of R.Q. after DIEDI administration may account, at least partially, for the hypoglycaemic effect observed. It is therefore reasonable to conclude that in diabetic animals DIEDI improves the peripheral utilization of glucose, when, as in diabetes, it is impaired.

TABLE 2. GLYCOGEN CONTENT OF NORMAL AND DIABETIC RATS
(g per cent of fresh tissue)

The animals, kept starved 11 hr before being killed, were injected intraperitoneally with 0·4 g/kg of DIEDI and fed by gastric probe with 7·5 g/kg of glucose, 4 hr and 2 hr before respectively.

Normal rats			Diabetic rats				Diabetic rats treated with DIEDI (0·4 g/kg)			
No.	Liver	Muscle	No.	Blood sugar (mg/100 ml)	Liver	Muscle	No.	Blood sugar	Liver	Muscle
1	1·480	0·398	1	496	1·680	0·069	1	499	0·263	0·119
2	2·349	0·381	2	456	0·660	0·056	2	439	0·553	0·085
3	1·420	0·338	3	756	0·680	0·061	3	405	0·425	0·102
4	1·300	0·236	4	410	1·770	0·270	4	330	0·347	0·146
5	0·976	0·260	5	530	0·270	0·040	5	320	1·789	—
6	1·390	0·240	6	535	0·730	0·177	6	385	0·458	0·246
			7	776	0·161	0·046	7	446	1·847	0·215
			8	583	0·315	0·068	8	300	0·915	0·029
			9	330	2·068	0·259	9	467	0·341	—
Average	1·485	0·308			0·926	0·116			0·770	0·134

Since the results obtained in *in vitro* experiments have been inconclusive, it is possible that DIEDI may exert its action *in vivo* not directly, but after its transformation in some other substance, or by activating some system which is ineffective in the *in vitro* conditions.

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