Effects of 5-Methylpyrazole-3-Carboxylic Acid on Adipose Tissue

II. Antilipolytic and Hypoglycemic Effects *in Vivo*

E. R. Froesch, M. Waldvogel, U. A. Meyer, A. Jakob, and A. Labhart

Metabolic Unit, Department of Medicine, University of Zürich, Switzerland

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SUMMARY

5-Methylpyrazole-3-carboxylic acid stimulated the incorporation of labeled glucose into adipose tissue *in vivo* without stimulating the incorporation of labeled glucose into glycogen of the diaphragm and without increasing the glucose turnover of normal rats to any significant degree. Nicotinic acid shared with 5-methylpyrazole-3-carboxylic acid these effects on adipose tissue metabolism. 5-Methylpyrazole-3-carboxylic acid blocked the rise of the plasma free fatty acids and partially prevented the rise of the blood sugar in acutely insulin-deficient rats due to the maintenance of a normal utilization of glucose by the tissues (muscle) despite insulin deficiency. After prolonged treatment with 5-methylpyrazole-3-carboxylic acid an escape phenomenon was observed. 5-Methylpyrazole-3-carboxylic acid still acutely depressed the level of free fatty acids, but the effect was short lived. 5-Methylpyrazole-3-carboxylic acid stimulated incorporation of glucose into glycerides of adipose tissue more markedly and more rapidly in pretreated than in normal rats, but the fatty acids and glycerides synthesized from glucose left the tissue again more rapidly.

5-Methylpyrazole-3-carboxylic acid is a potent antilipolytic drug (1–4). Its antilipolytic activity on adipose tissue *in vitro* is about equal to that of nicotinic acid (4). Due to its long half-life, 5-methylpyrazole-3-carboxylic acid is many times as active as nicotinic acid when administered *in vivo* (G. C. Gerritsen and W. E. Dulin, personal communications). The mode of action of these drugs has not yet been elucidated.

We have tested the antilipolytic effects of 5-methylpyrazole-3-carboxylic acid in rats *in vivo* and were struck by the marked insulin-like effects which this drug exerts on glucose metabolism of adipose tissue. 5-Methylpyrazole-3-carboxylic acid stimulates the incorporation of ^{14}C-labeled glucose into adipose tissue as markedly as insulin, whereas it is without any effects on the incorporation of glucose into glycogen of the diaphragm. The results reported here help to explain the acute blood sugar lowering effects of 5-methylpyrazole-3-carboxylic acid (1). They also shed some doubt on the usefulness of these drugs in the long-term management of metabolic disorders such as diabetes mellitus and hyperlipidemia.

METHODS

Glucose was measured enzymically (5). Free fatty acids were extracted and titrated according to Gordon (6). The method used for the extraction, isolation, and counting procedures of total lipids, fatty acids after hydrolysis of the total lipids, and of glycogen of adipose tissue and diaphragm have been published elsewhere in detail (3, 7). D-Mannoheptulose was a gift of Dr. E. Simon, Rehovoth, Israel. Antiinsulin serum was produced as described earlier, and it had an insulin-neutralizing capacity of approximately 0.5 unit/ml as determined in the adipose tissue assay (8). 5-Methylpyrazole-3-carboxylic acid was a gift of Drs. G. C. Gerritsen and W. E. Dulin of the Upjohn Company, Kala-

mazoo, Michigan. Nicotinic acid was purchased from Merck, Germany. The albumin fraction V, lot No. 65/08-10, was prepared by the Blutspendedienst of the Swiss Red Cross according to the method of Kistler and Nitschmann (9). It was devoid of any insulin-inhibitory or insulin-like activity (8).

Experimental procedure. Male purebred Osborne-Mendel rats weighing 95–105 g were found to be well suited for testing the effects of insulin-like material *in vivo* (10). The day preceding the experiment they were changed from a diet of pellets to a diet containing oat flakes, carrots, dry bread, and 10% sucrose in the water. The food was removed at 7 A.M. on the day of the experiment and the water containing sucrose was replaced by plain water. The experiment was started at 1 P.M. The rats were anesthesized with 10 mg of diethylaminoallylisopropylbarbituric acid administered subcutaneously. After 1 hr, 1 ml of physiologic saline, containing 5 μC of glucose-6-^{14}C and the test substances, as indicated in the legend of the tables and figures, was injected intravenously into the tail vein. The rats were killed at appropriate time intervals by decapitation. Neck blood was collected on ice into sodium oxalate and centrifuged; the plasma was frozen. The diaphragm was excised, weighed, and digested in boiling 30% KOH. The epididymal fat pads were weighed, and

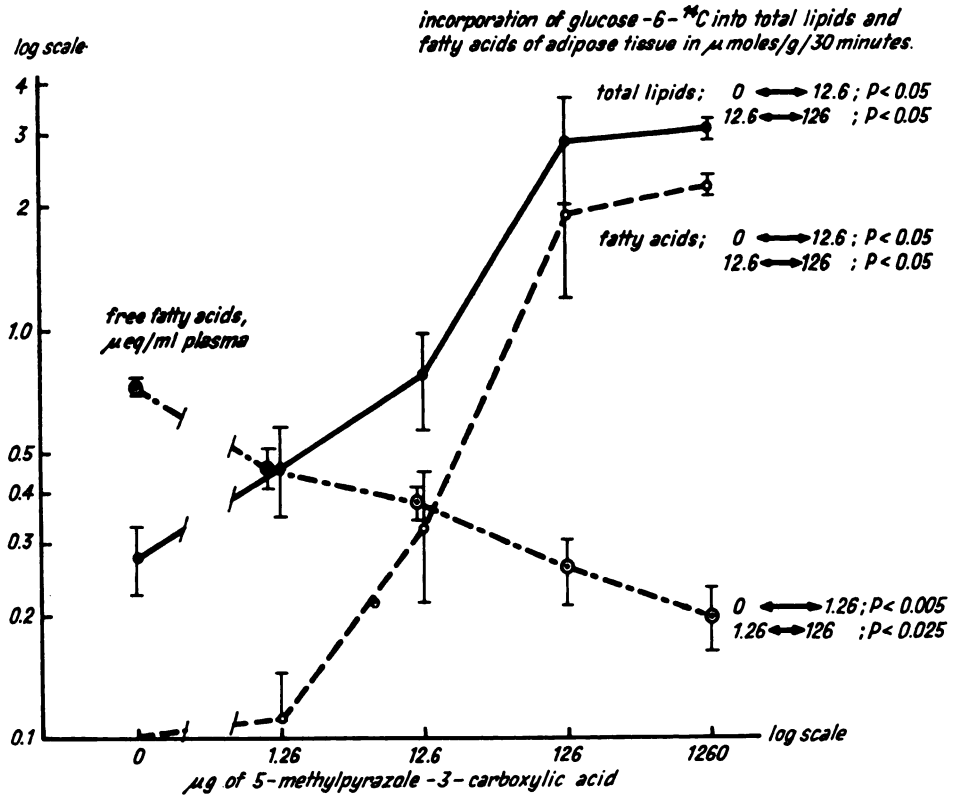

FIG. 1. *Effects of 4 doses of intravenously administered 5-methylpyrazole-3-carboxylic acid on plasma free fatty acids and on the incorporation of glucose-6-^{14}C into total lipids and fatty acids of adipose tissue*

5-Methylpyrazole-3-carboxylic acid was dissolved in physiologic saline and 1 ml was administered intravenously together with a tracer dose of glucose-6-^{14}C. The rats were killed after 30 min. The means of the results of 4 rats and the SEM are given. The specific activity of the blood glucose at the time of sacrifice was used to express the results of the incorporation into adipose tissue in terms of micromoles of glucose-6-^{14}C per gram per 30 min.

the total lipids were extracted in chloroform–methanol 2:1. The experimental procedure was modified in the experiment represented in Fig. 3 as indicated in the legend.

RESULTS

The relationship between the dose of 5-methylpyrazole-3-carboxylic acid and its effects on plasma free fatty acids and on the incorporation of glucose-6-^{14}C into total lipids and fatty acids of adipose tissue is shown in Fig. 1. 1.26 μg of the drug significantly decreased the level of free fatty acids and stimulated the incorporation of carbon-14 into the fat pads. The stimulation of adipose tissue metabolism by 5-methylpyrazole-3-carboxylic acid became significant at a dose level of 12.6 μg of 5-methylpyrazole-3-carboxylic acid per rat. Maximal stimulation of the incorporation of glucose-6-^{14}C into fatty acids of the fat pads occurred at a dose of 126 μg per rat and was 20-fold above baseline.

In order to investigate whether or not the stimulatory action of 5-methylpyrazole-3-carboxylic acid on glucose metabolism of adipose tissue was shared by compounds of similar configuration, nicotinic acid was administered intravenously to rats under the same experimental conditions (Table 1). A 1.2-mg amount of nicotinic acid significantly decreased the plasma level of free fatty acids. Stimulation of the adipose tissue metabolism was very marked already at a dose of 126 μg of nicotinic acid per rat. The drug had no effect on the incorporation of glucose-6-^{14}C into the glycogen of the diaphragm.

The time course of the effects of 5-methylpyrazole-3-carboxylic acid is shown in Fig. 2. Rats were killed 15, 30, and 60 min after intravenous injection of this drug. During the whole period the level of plasma free fatty acids was significantly decreased by 5-methylpyrazole-3-carboxylic acid. The blood sugar was not significantly affected by the drug, whereas adipose tissue metabolism was again stimulated. The half-life of blood glucose was 30 min in the group of rats injected with saline, and 25 min in the group receiving 5-methylpyrazole-3-carboxylic acid, but this difference was not statistically significant.

TABLE 1

Effects of two doses of intravenously administered nicotinic acid on the level of free fatty acids and on the incorporation of intravenously injected glucose-6-^{14}C into total lipids and fatty acids of adipose tissue and into glycogen of the diaphragm

Nicotinic acid was dissolved in saline and 1 ml was injected into the tail vein. The rats were killed after 30 min. The means of the results obtained in 5 rats and the standard error of the means are given. Students *t* test was applied for statistical analysis. The results which are expressed as micromoles of glucose-6-^{14}C incorporated per gram per hour were obtained by dividing the counts per minute in the respective metabolic indices by the specific activity of the blood glucose at the time of sacrifice. P^* = statistical analysis between control group receiving saline and the group receiving 1.2 mg of nicotinic acid.

Drug and dosage, i.v.	Incorporation of glucose-6-^{14}C (μmoles/g/30 min into adipose tissue			Free fatty acids (μeq/ml plasma)
	Total lipids	Fatty acids	Diaphragm glycogen	
NaCl, 0.9%, 1 ml	0.23 ±0.06 $P < 0.005$	0.07 ±0.04 $P < 0.01$	0.06 ±0.01 $P > 0.25$	0.73 ±0.04* *$P < 0.0005$
Nicotinic acid, 0.12 mg	3.07 ±0.68 $P > 0.10$	2.13 ±0.58 $P > 0.10$	0.05 ±0.01 $P > 0.10$	0.70 ±0.29
Nicotinic acid, 1.2 mg	4.91 ±1.14	4.07 ±1.15	0.07 ±0.01	0.28 ±0.02*

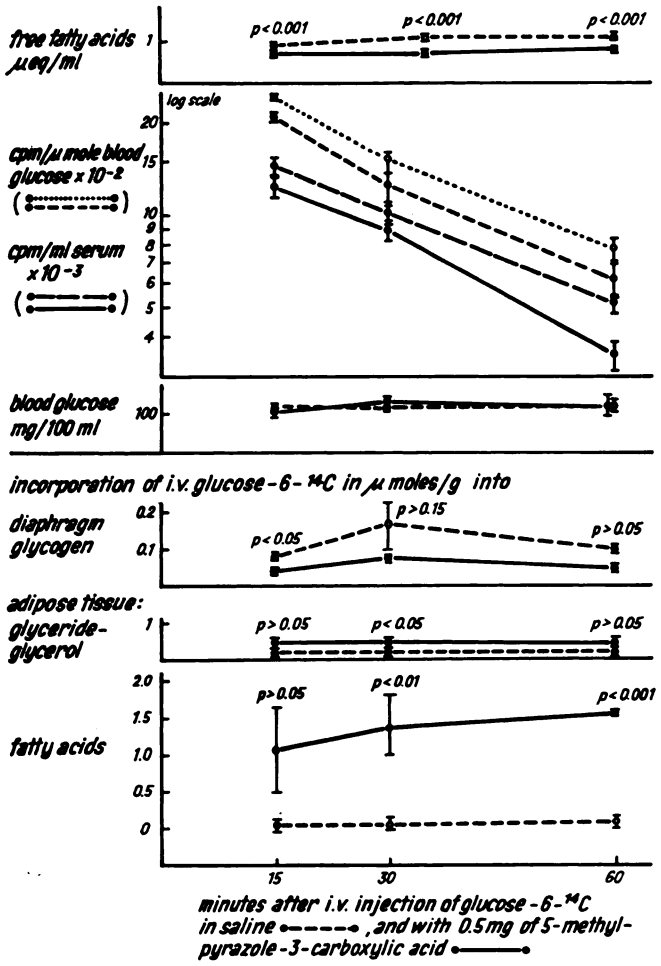

FIG. 2. *Effects of intravenously administered 5-methylpyrazole-3-carboxylic acid on blood glucose, plasma free fatty acids, and incorporation of glucose-6-^{14}C into tissues as a function of time*

5-Methylpyrazole-3-carboxylic acid, 0.5 mg in 1 ml of saline, was injected intravenously together with a tracer dose of glucose-6-^{14}C. Control rats were given 1 ml of saline. The rats were sacrificed after 15, 30, and 60 min, respectively. The means of the results of 4 rats and the SEM are given. The specific activity of the blood glucose at the half-time between injection and sacrifice was used to express the results in terms of micromoles of glucose-6-^{14}C incorporated per gram.

A clear-cut blood sugar-lowering effect of 5-methylpyrazole-3-carboxylic acid was observed in rats rendered acutely diabetic with anti-insulin serum. It may be seen in Table 2 that 5-methylpyrazole-3-carboxylic acid did not affect the blood sugar of rats receiving an albumin solution, whereas it prevented the blood sugar rise following the injection of anti-insulin serum. It also blocked the rise of the plasma level of the free fatty acids.

This antidiabetic effect of 5-methylpyrazole-3-carboxylic acid was further analyzed in an experiment represented in Table 3. A tracer-dose of labeled glucose-6-^{14}C was injected simultaneously with the drug to permit a better analysis of the metabolic situation. Again the rise of blood sugar following the injection of anti-insulin serum was blocked by 5-methylpyrazole-3-carboxylic acid. The drug did not affect the rate of disappearance of labeled glucose from the blood in the group of rats injected with albumin. Under the influence of anti-

TABLE 2

Effects of 5-methylpyrazole-3-carboxylic acid, insulin, antiinsulin serum alone and with 5-methylpyrazole-3-carboxylic acid on blood glucose and free fatty acids

One milliliter of saline with 3 g of albumin/100 ml or of undiluted dialyzed anti-insulin guinea pig serum was injected into the tail vein with or without 0.5 mg of 5-methylpyrazole-3-carboxylic acid. The dose of insulin was 6 milliunits/per rat. The rats were killed 30 min after the intravenous injection. The means of the results obtained in 4 rats and the SEM are given.

	Administration 30 minutes prior to sacrifice of				
Metabolic index	Albumin	Albumin + insulin	Albumin + 5-methylpyrazole-3-carboxylic acid	Anti-insulin serum	Anti-insulin serum + 5-methylpyrazole-3-carboxylic acid
Blood glucose (mg%)	139.6 ±3.8	109.2 ±7.0	126.0 ±5.6	203.8 ±1.9	145.6 ±3.4
P versus albumin <	—	0.01	0.10	0.005	0.20
Free fatty acids (μeq/ml)	1.262 ±0.012	0.914 ±0.033	1.066 ±0.012	1.580 ±0.031	0.980 ±0.020
P versus albumin <	—	0.0005	0.0005	0.0005	0.0005

TABLE 3

Comparison of the effects of 5-methylpyrazole-3-carboxylic acid with those of insulin on the metabolism of intravenously administered glucose-6-^{14}C in normal and acutely diabetic rats

Insulin deficiency was induced by the intravenous injection of 1 ml of anti-insulin serum diluted 1:2. The means of the results obtained in 4 rats and the SEM are given. The specific activity of the blood glucose at the time of sacrifice was used to express the results of the incorporation in terms of glucose-6-^{14}C/g/30 min. Student's *t*-test was applied. Code for statistical treatment:

versus albumin: $a = P < 0.005$; $b = P < 0.01$; $c = P < 0.05$

versus anti-insulin serum: $x = P < 0.005$; $y = P < 0.05$

					Incorporation of glucose-6-^{14}C in μmoles/g/30 min into adipose tissue		
Drug and dosage, i.v.	Plasma free fatty acids (μeq/ml)	Blood glucose (mg/100 ml)	Cpm of blood glucose/ml plasma	Cpm/μmole blood glucose	Fatty acids	Glyceride-glycerol	Diaphragm glycogen
3 g% albumin, 1 ml	1.08 ±0.03	134 ±5	9185 ±512	1234 ±59	0.46 ±0.12	1.14 ±0.61	0.80 ±0.20
3 g% albumin + 0.5 mg 5-methylpyrazole-3-carboxylic acid	0.75[a] ±0.00	112 ±15	8514 ±700	1374 ±120	5.32[b] ±1.36	2.50 ±0.69	0.34 ±0.17
3 g% albumin + 6 mU of insulin	1.16 ±0.03	137 ±7	5290[a] ±410	708[a,x] ±87	3.40[c] ±0.86	3.56[c] ±0.56	7.01[b] ±1.76
Anti-insulin serum, dil. 1:2, 1 ml	1.38[a] ±0.00	191[b] ±13	15044[a] ±887	1437 ±144	0.09[c] ±0.03	0.29 ±0.09	0.06[b] ±0.01
Anti-insulin serum, dil. 1:2 + 0.5 mg 5-methylpyrazole-3-carboxylic acid	0.75[a,x] ±0.00	153 ±17	9202[x] ±150	1114[x] ±99	4.07[a,x] ±0.46	1.55 ±0.68	0.10[b] ±0.04

TABLE 4

Potentiation of the insulin effects on adipose tissue metabolism by 5-methylpyrazole-3-carboxylic acid after induction of the acute diabetic syndrome by mannoheptulose

Rats were rendered diabetic by the subcutaneous injection of 200 mg of mannoheptulose alone or together with 1.26 mg of 5-methylpyrazole-3-carboxylic acid. One hour later 6 milliunits of insulin were injected intravenously together with a tracer dose of glucose-6-^{14}C and the rats were killed 5, respectively, 15 min thereafter. The specific activity of the blood glucose at the time of sacrifice was used to express the results of the incorporation in terms of glucose-6-^{14}C per gram 30 minutes. The mean of the results obtained in 4 rats and the SEM are given. The *P* values (*t* test) give the statistical comparison of the results obtained in the rats injected with mannoheptulose alone and in those treated with mannoheptulose together with 5-methylpyrazole-3-carboxylic acid.

	Mannoheptulose, 200 mg, s.c. 1 hour prior to experiment					
Pretreatment:	–	+ 5-Methylpyrazole-3-carboxylic acid	*P*	–	+ 5-Methylpyrazole-3-carboxylic acid	*P*
Treatment: Metabolic index	Insulin, 6 mU i.v., sacrifice after 5 min			Insulin, 6 mU i.v., sacrifice after 15 min		
Plasma free fatty acids (μeq/ml)	0.94 ± 0.03	0.61 ± 0.00	<0.001	0.78 ± 0.03	0.48 ± 0.03	<0.001
Blood glucose (mg/100 ml)	340 ± 16	201 ± 9	<0.001	300 ± 34	228 ± 26	>0.05
Blood glucose (cpm/0.1 ml plasma)	2402 ± 92	2056 ± 27	<0.025	1705 ± 104	1461 ± 135	>0.10
Blood glucose (cpm/μmole)	1282 ± 261	1852 ± 83	>0.05	1031 ± 58	1164 ± 31	>0.05
Incorporation of glucose-6-^{14}C (μmoles/g) into adipose tissue						
Fatty acids	0.111 ± 0.009	0.378 ± 0.080	<0.025	0.289 ± 0.117	1.531 ± 0.173	<0.001
Glyceride-glycerol	0.295 ± 0.039	0.181 ± 0.036	<0.05	0.459 ± 0.119	0.187 ± 0.054	>0.05
Glycogen	0.025 ± 0.004	0.153 ± 0.014	<0.001	0.036 ± 0.013	0.556 ± 0.062	<0.001
Diaphragm glycogen	0.584 ± 0.297	0.604 ± 0.148	>0.49	1.692 ± 0.389	1.751 ± 0.328	>0.45

insulin serum the carbon-14 activity of the blood glucose remained significantly higher than in the control group injected with albumin. 5-Methylpyrazole-3-carboxylic acid administered together with anti-insulin serum reduced the carbon-14 activity of the blood sugar to the level observed in the control group injected with albumin alone. Insulin administered together with albumin significantly lowered both the total activity of the blood sugar as well as the specific activity of the blood glucose. 5-Methylpyrazole-3-carboxylic acid administered together with albumin or anti-insulin serum and anti-insulin serum administered alone did not significantly affect the specific activity of the blood glucose. 5-Methylpyrazole-3-carboxylic acid was as active as insulin in stimulating the incorporation of glucose-^{14}C into fatty acids and glyceride-glycerol of adipose tissue. The effects of 5-methylpyrazole-3-carboxylic acid on these metabolic indices were the same whether it was injected together with albumin or with anti-insulin serum. Anti-insulin serum administered alone inhibited both the incorporation of glucose-6-^{14}C into adipose tissue as well as that into glycogen

of the diaphragm. 5-Methylpyrazole-3-carboxylic acid was without any effect on the incorporation of labeled glucose into the glycogen of the diaphragm whereas insulin stimulated this metabolic index about 10-fold.

As shown in Table 4 the effects of insulin and of 5-methylpyrazole-3-carboxylic acid on glucose metabolism of adipose tissue are additive. Rats were rendered diabetic by the subcutaneous injection of 200 mg of mannoheptulose. In a second group of rats 5-methylpyrazole-3-carboxylic acid was injected subcutaneously together with mannoheptulose. One hour later, at the peak of the blood sugar, 6 milliunits of crystalline insulin were administered intravenously. The rats were killed 5 or 15 min after the intravenous administration of insulin and glucose-6-^{14}C. Insulin decreased the level of free fatty acids in both groups of rats. Their rise to diabetic values was prevented by pretreatment with 5-methylpyrazole-3-carboxylic acid. The incorporation of glucose-6-^{14}C into the glycogen of the diaphragm was stimulated by insulin to the same extent in both groups of rats. The incorporation of glucose-6-^{14}C into total lipids, fatty acids, and glycogen of the fat pads was significantly greater in the rats pretreated with 5-methylpyrazole-3-carboxylic acid, whereas the incorporation into glyceride-glycerol was reduced. The rise of the blood sugar due to mannoheptulose was partially prevented by the pretreatment with 5-methylpyrazole-3-carboxylic acid. The half-life of glucose calculated from the carbon-14 activity of the blood glucose was 22 min in both groups of rats. The decay of the specific activity of blood glucose was steeper in the group pretreated with 5-methylpyrazole-3-carboxylic acid. The hepatic glucose release was calculated to amount to 72 mg/hr in the mannoheptulose group and to 90 mg/hr in the group of rats pretreated with 5-methylpyrazole-3-carboxylic acid.

The last experiment was performed to test the effects of a long-term treatment with 5-methylpyrazole-3-carboxylic acid. Rats were given 1 mg of 5-methylpyrazole-3-carboxylic acid subcutaneously b.i.d. The control group received 1 ml of saline b.i.d. Six hours after the tenth injection of 5-methylpyrazole-3-carboxylic acid 1 mg of the drug was administered intravenously together with a tracer dose of glucose-6-^{14}C. The rats were killed after 10, 60, and 120 min. The initial level of the plasma free fatty acids was significantly lower in the control group than in the rats pretreated with 5-methylpyrazole-3-carboxylic acid. In both groups the plasma level of free fatty acids fell below 3 μeq/ml within 10 min after the injection of 5-methylpyrazole-3-carboxylic acid and it remained low for 60 min. At 120 min the control group still exhibited a low level of free fatty acids, whereas it had risen to almost the initial level in the rats pretreated with 5-methylpyrazole-3-carboxylic acid. The incorporation of carbon-14 into the fatty acids and glyceride-glycerol of adipose tissue was quite different in both groups of rats. Whereas carbon-14 continued to be incorporated into adipose tissue of rats pretreated with saline during the entire period of observation, it rose to a sharp peak in the group pretreated with 5-methylpyrazole-3-carboxylic acid 10 min after the injection of the drug. Thereafter the carbon-14 activity in the fatty acids and in glyceride-glycerol decreased to levels well below those observed in the control group at 120 min. The carbon-14 activity of the plasma free fatty acids was significantly higher in the rats pretreated with the drug than in the control rats.

DISCUSSION

The metabolic effects of intravenously administered 5-methylpyrazole-3-carboxylic acid were investigated and found to be 2-fold. This drug very effectively inhibits lypolysis of adipose tissue *in vitro* and stimulates the glucose uptake and incorporation of ^{14}C-labeled glucose into fatty acids to a slight extent (3, 4). Although both these effects are also exerted by insulin, the degree of the stimulation of glucose uptake *in vitro* is of an entirely different order of magnitude. Maximal stimulation of glucose uptake by 5-methylpyrazole-3-carboxylic acid *in vitro* was

50% above the baseline, whereas insulin may stimulate glucose uptake between 4- and 10-fold (3, 4).

5-Methylpyrazole-3-carboxylic acid stimulated the glucose metabolism of adipose tissue *in vivo* much more markedly than *in vitro*. After the intravenous administration of 5-methylpyrazole-3-carboxylic acid and glucose-6-^{14}C the carbon-14 activity in the total lipids increased in a linear manner (Figs. 2 and 3) indicating that net fat synthesis from glucose was occurring. This drug stimulated the incorporation of glucose-6-^{14}C into fatty acids and glyceride-glycerol of adipose tissue to about the same extent as 6 milliunits of insulin administered in a single intravenous injection (Table 3). Whereas the effect of intravenously administered insulin on the glucose uptake of adipose tissue did not last longer than 30 minutes (10), 5-methylpyrazole-3-carboxylic acid led to a prolonged stimulation. In the experiment represented in Fig. 3, the incorporation of glucose-6-^{14}C into fatty acids of adipose tissue seemed to increase in a linear fashion with time up to 120 min after the injection of 5-methylpyrazole-3-carboxylic acid. Rats pretreated with 5-methylpyrazole-3-carboxylic acid 1 hr prior to the intravenous administration of glucose-6-^{14}C still incorporated significantly greater amounts of glucose carbon-14 into fatty acids than did the control rats (Table 4).

As shown in Table 1 nicotinic acid shares with 5-methylpyrazole-3-carboxylic acid not only the antilipolytic effects, but also the stimulatory effects on glucose metabolism of adipose tissue. 1.26 mg of nicotinic acid per 100 g rat stimulated the glucose metabolism of adipose tissue to about the same extent as a maximal dose of 5-methylpyrazole-3-carboxylic acid.

These very marked effects of 5-methylpyrazole-3-carboxylic acid and of nicotinic acid on glucose metabolism of adipose tissue are in sharp contrast to the complete lack of any effect of these drugs on the metabolism of the diaphragm. The incorporation of glucose-6-^{14}C into the glycogen of this tissue was neither stimulated by 5-methylpyrazole-3-carboxylic acid nor by nicotinic acid. The difference between 5-methylpyrazole-3-carboxylic acid and insulin in this respect is particularly obvious in the experiment represented in Table 3. Whereas 6 milliunits of insulin stimulated the incorporation of glucose carbon-14 into the glycogen of the diaphragm from 0.80 to 7.01 μmoles/g/30 min, 5-methylpyrazole-3-carboxylic acid had a slightly inhibitory but nonsignificant effect.

Despite these marked effects of 5-methylpyrazole-3-carboxylic acid on glucose metabolism of adipose tissue and despite the inhibition of fatty acid release this drug did not significantly lower the blood sugar of normal rats (Tables 2 and 3, Fig. 2). The half-life of blood glucose in Osborne-Mendel rats weighing 100 g is between 18 and 30 min (10) (Fig. 2). It is impossible to quantitatively assess the role of adipose tissue, muscle, and other tissues in overall glucose turnover. However, the experiments represented here seem to indicate that a marked stimulation of glucose metabolism of adipose tissue by 5-methylpyrazole-3-carboxylic acid which is comparable to maximal insulin stimulation did not increase the overall glucose turnover to a significant degree. Therefore, one must assume that the role played by adipose tissue in the overall glucose utilization is very small. The same conclusion has been reached by others who studied the glucose metabolism of the rat *in vivo* (11, 12). The reason for the overestimation of the quantitative role played by adipose tissue in overall glucose metabolism stems from *in vitro* studies. Whereas adipose tissue *in vivo* did not appear to incorporate significant quantities of labeled glucose into fatty acids, the same tissue incubated *in vitro* exhibited net fatty acid synthesis from labeled glucose and became more active during the course of a prolonged incubation (13–15). This artificially elevated basal glucose uptake of adipose tissue incubated *in vitro* may also be the reason why 5-methylpyrazole-3-carboxylic acid is much less active *in vitro* than *in vivo*.

5-Methylpyrazole-3-carboxylic acid effectively prevented the increase of the blood sugar induced by the cessation of in-

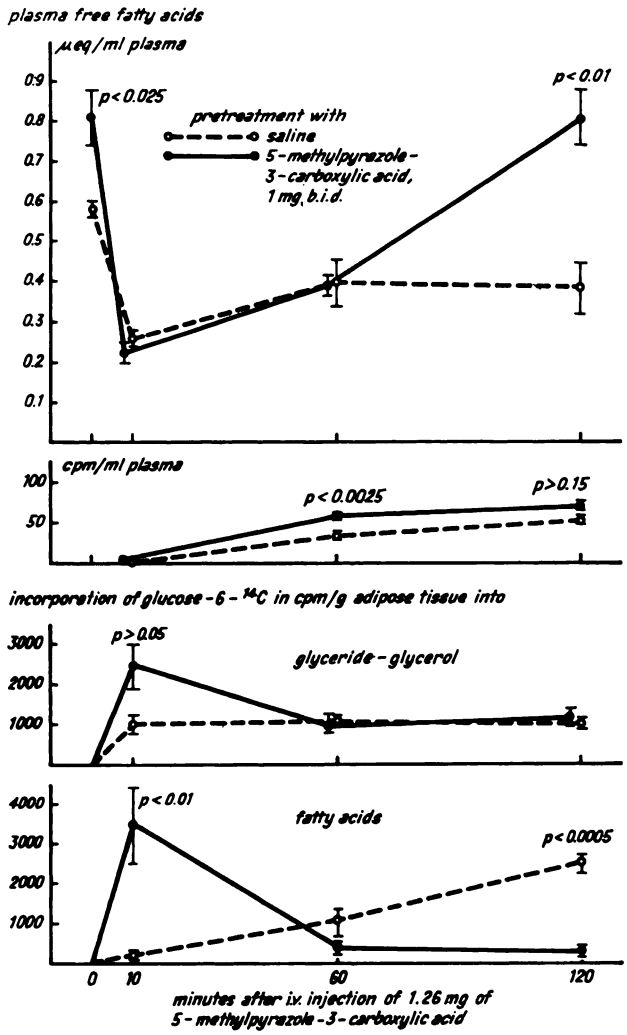

FIG. 3. *Effects of 5-methylpyrazole-3-carboxylic acid on glucose metabolism and lipolysis of adipose tissue after pretreatment with this drug for 6 days*

Twenty rats were injected subcutaneously with 1 mg of 5-methylpyrazole-3-carboxylic acid b.i.d. during 6 days. A control group received 1 ml of saline b.i.d. At 5 hr after the last subcutaneous injection, the rats were anesthetized and an intravenous dose of 1.26 mg of 5-methylpyrazole-3-carboxylic acid was administered together with glucose-6-^{14}C. Five rats of each group were killed after 10, 60, and 120 min, respectively. The means of the results in 5 rats and the SEM are given.

sulin secretion by mannoheptulose (16) or by the binding and blocking of insulin by intravenously administered anti-insulin serum. The antidiabetic effects of 5-methylpyrazole-3-carboxylic acid were analyzed in the experiments represented in Tables 3 and 4. The acute diabetogenic effects of both mannoheptulose and anti-insulin serum were clearly due to the cessation of glucose outflow into tissues as indicated by the carbon-14 activity of the blood glucose which remained considerably higher than in the control rats. Hepatic overproduction of unlabeled glucose did not seem to come into play within this short period of insulin lack, since the specific activity of blood glucose was only slightly lower in the diabetic than in the nondiabetic rats.

In analogy to the aforementioned minor role of adipose tissue in overall glucose metabolism of normal rats, it seems unlikely that the stimulation of adipose tissue metabolism by 5-methylpyrazole-3-carboxylic acid was responsible for the prevention of hyperglycemia in the rats in which diabetes was acutely induced by anti-insulin serum or mannoheptulose. It must rather be assumed that glucose oxidation by muscle was maintained at a normal level despite the absence of insulin. Maintenance of the normal glucose utilization by muscle in the face of insulin lack was probably due to the unavailability of free fatty acids, the level of which was held low by 5-methylpyrazole-3-carboxylic acid. The apparently normal glucose oxidation by muscle of the acutely diabetic rats treated with 5-methylpyrazole-3-carboxylic acid was not reflected in a concomitant increase of glucose-6-^{14}C incorporation into glycogen. This is to be expected on the basis of a number of investigations which show that glycogen synthesis in muscle does not correlate with the rate of glycolysis and of glucose oxidation (17, 18). Inhibition of glycolysis and glucose oxidation by a number of substrates is not parallel to changes of the incorporation of glucose into glycogen (17–20).

Our results demonstrate that a level of free fatty acids above normal considerably decreased overall glucose utilization *in vivo*. This effect was independent of insulin and could be reversed by lowering the level of free fatty acids by 5-methylpyrazole-3-carboxylic acid.

Pavle, Issekutz, and Miller (21) have shown that nicotinic acid increased the glucose turnover by lowering the level of free fatty acids only in normal and not in pancreatectomized dogs 48 hours after insulin withdrawal. These dogs were severely diabetic in contrast to the acute insulin deficiency induced in our rats. A prolonged insulin deficiency leads to changes of hexokinase activity (23) and of the glucose carrier system in muscle (22–24) and in adipose tissue (25). The rate of glycolysis of muscle may then become independent of the level of free fatty acids.

In the last experiment presented in Fig. 3 we made an interesting observation which cannot yet be interpreted. It demonstrates that rats treated over a period of 5 days with large doses of 5-methylpyrazole-3-carboxylic acid develop mechanisms by which they may escape the antilipolytic effect of 5-methylpyrazole-3-carboxylic acid. The plasma level of the free fatty acids was significantly higher in rats pretreated during 5 days with 5-methylpyrazole-3-carboxylic acid than in the control group pretreated with saline 6 hr after the last injection. The acute effect of the drug on the plasma free fatty acids was the same in both groups of rats, but they rose to the initial level within 120 min in the group pretreated with 5-methylpyrazole-3-carboxylic acid whereas they remained low during the whole period of observation in the control group. Stimulation of the incorporation of labeled glucose into fatty acids and glyceride-glycerol of adipose tissue was more marked acutely after the injection of 5-methylpyrazole-3-carboxylic acid in the rats premedicated with this drug than in the control rats. However, the glycerides synthesized under the influence of 5-methylpyrazole-3-carboxylic acid seemed to be hydrolyzed and released by the tissue very rapidly after their synthesis. The carbon-14 activity in the free fatty acids of the plasma was significantly higher in the rats pretreated with 5-methylpyrazole-3-carboxylic acid than in the control rats 60 minutes after the intravenous injection of this drug. These data indicate that under the chronic administration of 5-methylpyrazole-3-carboxylic acid both synthesis of glycerides from glucose as well as hydrolysis of freshly synthesized glycerides are activated to a marked degree. The net result of these two phenomena is an actual elevation of the free fatty acid concentration above the control level. According to these results the chronic administration of this type of antilipolytic drug would result in the contrary of what one expects of a therapy with antilipolytic drugs, i.e., a reduction of the level of free

fatty acids leading to a reduction of the level of the triglycerides. It will be of considerable interest to find out whether similar phenomena occur in men upon prolonged treatment with 5-methylpyrazole-3-carboxylic acid and similar drugs. Treatment of diabetic subjects with nicotinic acid over prolonged periods of time did not ameliorate, but rather aggravated, the metabolic condition making necessary an increase of the insulin dose (26). This clinical observation might be explained by our findings of escape mechanisms from the effects of antilipolytic drugs resulting in elevated rather than decreased levels of free fatty acids. According to Smith, Wagner, and Gerritsen (27) this phenomenon is not due to an accelerated metabolism and excretion of 5-methylpyrazole-3-carboxylic acid. The mechanisms involved in this escape phenomenon are presently under investigation in our laboratory.

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