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3-Mercaptopicolinic Acid, An Inhibitor of Gluconeogenesis

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1. 3-Mercaptopicolinic acid (SK&F 34288) inhibited gluconeogenesis *in vitro*, with lactate as substrate, in rat kidney-cortex and liver slices. 2. In perfused rat livers, gluconeogenesis was inhibited when lactate, pyruvate or alanine served as substrate, but not with fructose, suggesting pyruvate carboxylase or phosphoenolpyruvate carboxylase as the site of inhibition. No significant effects were evident in O_2 consumption, hepatic glycogen, urea production, or [lactate]/[pyruvate] ratios. 3. A hypoglycaemic effect was evident *in vivo* in starved and alloxan-diabetic rats, starved guinea pigs and starved mice, but not in 4h-post-absorptive rats. 4. In the starved rat the hypoglycaemia was accompanied by an increase in blood lactate. 5. A trace dose of [^{14}C]lactate *in vivo* was initially oxidized to a lesser extent in inhibitor-treated rats, but during 90min the total CO_2 evolved was slightly greater. The total amount of the tracer oxidized was not significantly different from that in the controls.

The uncontrolled nature of gluconeogenesis in diabetes mellitus makes it desirable to develop compounds which modulate this process in order to understand better the biochemistry involved and the importance of gluconeogenesis in contributing to the hyperglycaemia of diabetes.

A variety of compounds possessing different mechanisms of action have been shown to inhibit gluconeogenesis (Haeckel, 1972). However, a positive correlation between the inhibition of gluconeogenesis *in vitro* and the manifestation of this effect *in vivo*, as measured by hypoglycaemia, has not always been readily demonstrable.

Our investigations, dealing with the effects of various heterocyclic acids on gluconeogenesis, have revealed that 3-mercaptopicolinic acid (SK&F 34288) is an effective inhibitor of gluconeogenesis, capable of inducing hypoglycaemia in starved or alloxan-diabetic animals. The experimental evidence pertaining to this finding is the subject of the present paper.

Experimental

Animals

Charles River male rats, maintained on Purina Lab Chow and water *ad libitum* and weighing 200–250g before starvation, supplied livers or kidneys for experiments *in vitro* or were used for experiments *in vivo*. Alloxan-diabetes was induced by the intravenous administration of recrystallized alloxan monohydrate at a dose of 60mg/kg to non-starved rats. Rats with a non-starvation blood glucose value in excess of 250mg/100ml 48h after the administration of alloxan were considered diabetic and were used experimentally the next day after starvation for 12h. Normal post-absorptive rats were starved for 4h before test but were permitted free access to water.

Mice were Charles River males, maintained on Purina Lab Chow and water *ad libitum*, weighing approx. 25g before starvation.

Male guinea pigs of the random-bred English short-hair strain were maintained on Purina guinea-pig pellets, fresh lettuce, and water *ad libitum* and weighed 175–225g before starvation. The guinea pigs were killed by decapitation and blood was collected from the severed vessels for glucose determinations.

Chemicals

All chemicals were the best grade commercially obtainable.

Analytical procedures

In the experiments *in vitro* and liver perfusions, glucose was assayed by the glucose oxidase method (Hugget & Nixon, 1957) (enzyme from Boehringer Mannheim, New York, N.Y., U.S.A.). In the experiments *in vivo*, whole-blood glucose was assayed by the micro-ferriferrocyanide or the modified glucose oxidase procedure for the Technicon AutoAnalyzer (Technicon AutoAnalyzer Methods Manual 1971,

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Technicon Corp., Tarrytown, N.Y., U.S.A.). Pyruvate and lactate were determined enzymically in $HClO_4$ extracts of whole blood or perfusion medium by the methods of Bücher *et al.* (1963) and Hohorst (1963) respectively.

After extraction by the procedure of Moody & Felber (1966), liver glycogen was determined by the anthrone method of Carroll *et al.* (1956). Urea N was determined by the method of Kaplan (1965).

O_2 consumption by the perfused liver was calculated by determining the afferent and efferent O_2 content of the perfusion media, by using an oxygen electrode (PHA 927 Gas Monitor; Radiometer, Copenhagen, Denmark).

Kidney slices

Kidneys obtained from 48h-starved rats were rinsed in cold 0.9% (w/v) NaCl, and slices were cut with a Stadie–Riggs slicer. The cortex was cut free of medullary tissue, rinsed for 5min in Krebs–Henseleit buffer low in bicarbonate (2.88mM), and then incubated in Krebs bicarbonate buffer, pH7.4 (Krebs *et al.*, 1963), for 2h under O_2+CO_2 (95:5) at 37°C with 10mM-lactate with or without 3-mercaptopicolinic acid dissolved in Krebs bicarbonate buffer added at zero time. Samples (2ml) of the incubation medium were deproteinized with 3M-$HClO_4$ and the concentration of glucose was determined in the clear supernatant.

Liver perfusions

Livers were obtained from rats starved overnight. Perfusions were carried out as described by Ruderman & Herrera (1968). The perfusion medium consisted of 125ml of Krebs bicarbonate buffer containing 0.154M-NaCl, 6mM-K^+, 3mM-Ca^{2+}, 2mM-PO_4^{3-}, 2mM-Mg^{2+}, 32mM-HCO_3^-, 4% fatty acid-poor bovine serum albumin fraction V (Miles Laboratories Inc., Kankakee, Ill., U.S.A.) and 0.2ml of sodium heparin (Lilly, Indianapolis, Ind., U.S.A.; 1000U.S.P. units/ml). Before use, the medium was filtered through a 0.45μm Millipore filter and the pH adjusted to 7.4 with 1.0M-NaOH. The perfusion flow rate was 40–60ml/min with a gas flow of O_2+CO_2 (95:5) at 3.3 litres/min. Perfusions were for 90min, and the perfusion medium was sampled at 0, 30, 60 and 90min or 0, 20, 40, 60 and 90min for the measurement of various parameters. The viability of the perfusion preparation was monitored by bile production.

[^{14}C]Lactate oxidation

A modification of the original method of Weinhouse & Friedmann (1951) was used to assess the effect of 3-mercaptopicolinic acid on the oxidation of L-[3-^{14}C]lactate (New England Nuclear Corp., Boston, Mass., U.S.A.). Rats starved for 48h were dosed (125mg/kg) orally with 3-mercaptopicolinic acid and 2h later were given intravenously a trace dose of L-[3-^{14}C]lactate [2.5μCi (0.44μmol)/rat] and immediately placed, individually, in a sealed metabolism chamber. The expired CO_2 was collected for three consecutive periods of 30min by passing the expired air through glass-beaded towers containing 2.5M-NaOH. The CO_2 was quantitatively precipitated from solution by the addition of 20% (w/v) $BaCl_2$. The precipitated $BaCO_3$ was filtered, washed, dried and weighed. A sample was counted for radioactivity by liquid-scintillation counting by using 0.4% (w/v) PPO (2,5-diphenyloxazole, Pilot Chemicals Inc., Watertown, Mass., U.S.A.) in toluene to which 300mg of Thixin-R (Baker Castor Oil Co., Bayonne, N.J., U.S.A.) was added for each 10ml of phosphor.

All statistical calculations were done by Student's *t* test (Snedecor & Cochran, 1967).

Results

Rat kidney-cortex slices

In the kidney-cortex slices, significant inhibition ($P<0.001$) of glucose production was obtained with 3-mercaptopicolinic acid at 0.1 and 0.01mM, but not at 0.001mM (Table 1). Although not shown, significant inhibition of glucose production by rat liver slices was also obtained at 1 and 0.1mM.

Rat liver perfusions

The isolated perfused liver obtained from starved rats was used to ascertain the effect of 3-mercaptopicolinic acid on gluconeogenesis when lactate, pyruvate, alanine, or fructose (all 10mM) served as substrate (Fig. 1). At 0.1mM, 3-mercaptopicolinic acid

Table 1. *Inhibition of gluconeogenesis with 3-mercaptopicolinic acid in rat kidney-cortex slices*

Male rats were starved for 48h for the kidney-cortex preparations. Further details are given in the text. Values are means±S.D., for the numbers of observations in parentheses. *Indicates statistical significance $P<0.001$, compared with controls.

Additions		Glucose (μmol/2h per g wet wt.)
[Lactate] (mM)	[3-Mercaptopicolinic acid] (mM)	Kidney-cortex slice
10	0	10.6±2.2 (6)
10	0.1	2.2±0.6 (5)*
10	0.01	4.4±1.7 (6)*
10	0.001	8.3±2.2 (6)

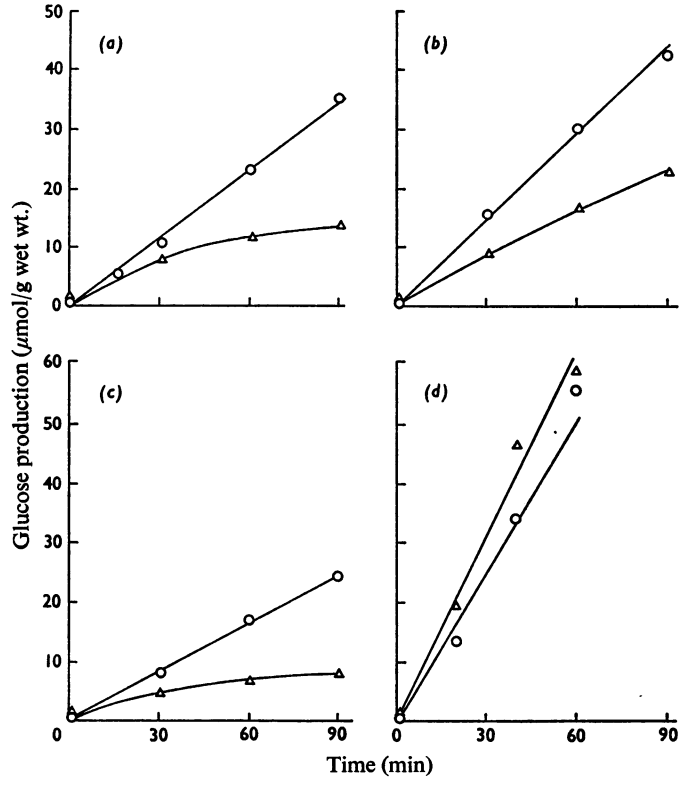

Fig. 1. *Effect of 3-mercaptopicolinic acid on glucose production by perfused rat livers*

Each point represents the mean glucose production by four to six control (○) or inhibitor-treated (△) livers. Substrate and inhibitor were added at zero time. See the text for additional details. (*a*) Lactate, (*b*) pyruvate, (*c*) alanine and (*d*) fructose were used as substrates.

significantly inhibited glucose production from lactate (60min $P<0.05$; 90min $P<0.01$), pyruvate (30 and 60min $P<0.01$; 90min $P<0.001$) and alanine (60min $P<0.01$; 90min $P<0.001$), but did not inhibit glucose production with fructose as substrate. Although not shown, 3-mercaptopicolinic acid (0.1mM) failed to inhibit glucose production when dihydroxyacetone (10mM) served as substrate. 3-Mercaptopicolinic acid was equally effective in inhibiting glucose production from lactate when it was added to the perfusion media 30min before lactate.

The perfusion media were sampled at various time-intervals for analysis of several parameters, and comparisons made between the control and experimental livers. The results shown in Table 2 are those obtained at 60min of perfusion, except for the glycogen values, which were obtained at 90min. The utilization of lactate or pyruvate, as determined by its disappearance from the media, was not impaired by the presence of 3-mercaptopicolinic acid. With pyruvate as substrate, its conversion into lactate was not affected by the inhibitor. Similarly, with lactate as substrate, its conversion into pyruvate was not affected. When alanine served as substrate, the production of lactate and pyruvate more than doubled in the livers perfused with 3-mercaptopicolinic acid, but this increase was statistically significant ($P<0.05$) only for pyruvate. When fructose was the substrate, there was no difference in the production of lactate or pyruvate between control and 3-mercaptopicolinic acid-perfused livers. The inhibitor did not significantly alter the [lactate]/[pyruvate] ratio with any of the substrates used, when compared with the respective controls.

With alanine as substrate, urea N production increased to approx. 0.40mg/g. This was naturally greater than with lactate, pyruvate or fructose as substrate. However, regardless of the substrate used, 3-mercaptopicolinic acid did not significantly influence urea N production.

O_2 consumption, measured at 60min of perfusion, was approx. 2.0μmol/min per g in all groups and was

Table 2. *Production or utilization of lactate, pyruvate, urea N, O_2 and glycogen by perfused livers*

Values are those obtained at 60 min minus zero-time values, except for liver glycogen which was the value obtained at 90 min. Values are the means ± S.D. of a minimum of four perfused livers. * Indicates statistical significance, $P<0.05$, compared with controls. A minus sign indicates utilization.

Substrate (10 mM)	Lactate (μmol/g)		Pyruvate (μmol/g)		[Lactate]/[Pyruvate]		Urea N (mg/g)		O_2 consumption (μmol/min per g)		Glycogen (mg/g)	
	Control	+3-Mercaptopicolinic acid	Control	+3-Mercaptopicolinic acid	Control	+3-Mercaptopicolinic acid	Control	+3-Mercaptopicolinic acid	Control	+3-Mercaptopicolinic acid	Control	+3-Mercaptopicolinic acid
Lactate	−77.3 ±30.4	−60.4 ±15.7	6.6 ±2.5	8.4 ±2.3	11.8 ±1.9	7.7 ±2.7	0.17 ±0.05	0.15 ±0.06	2.7 ±0.5	1.7 ±0.5	0.23 ±0.04	0.19 ±0.06
Pyruvate	43.4 ±3.6	39.9 ±3.5	−195.0 ±15.8	−166.3 ±15.5	0.23 ±0.03	0.24 ±0.00	0.12 ±0.04	0.19 ±0.03	2.4 ±0.3	2.0 ±0.3	0.20 ±0.04	0.19 ±0.02
Alanine	6.1 ±2.7	14.2 ±5.1	1.7 ±0.6	4.9 ±1.5*	4.2 ±3.5	3.4 ±2.1	0.42 ±0.09	0.36 ±0.06	1.9 ±0.3	1.8 ±0.2	0.17 ±0.02	0.19 ±0.05
Fructose	37.8 ±7.3	34.6 ±9.1	6.6 ±2.8	9.6 ±1.7	6.6 ±2.9	3.6 ±0.5	0.08 ±0.05	0.11 ±0.04	1.8 ±0.5	2.2 ±0.5	0.26 ±0.05	0.25 ±0.05†

† Mean ± S.D. of three livers.

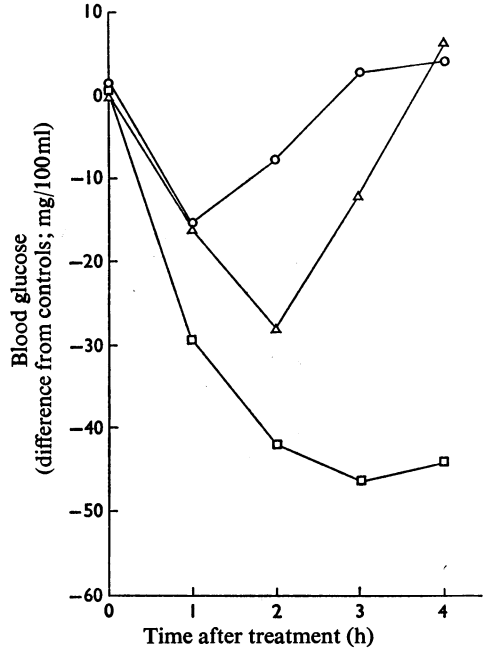

Fig. 2. *Hypoglycaemic effect of 3-mercaptopicolinic acid in starved rats*

Rats (eight per dose group) were starved for 18 h and given 3-mercaptopicolinic acid orally at doses of 37.5 (○), 75 (△) and 150 (□) mg/kg suspended in 1% (w/v) methyl cellulose. Repeated tail-vein blood samples were obtained from each rat at the indicated times.

not affected by the presence of 3-mercaptopicolinic acid in the perfusion media.

The glycogen content of the perfused livers, measured at the end of the perfusion experiment, was not statistically significantly different between control and 3-mercaptopicolinic acid-perfused livers, regardless of the substrate used.

Hypoglycaemic activity

Fig. 2 depicts the hypoglycaemic activity obtained with 3-mercaptopicolinic acid in rats starved for 18 h at oral doses of 37.5, 75 and 150 mg/kg. A significant ($P<0.001$) difference in blood glucose between control and treated groups was obtained with all doses 1 h after treatment, with the 75 and 150 mg/kg dose 2 h after treatment and with the 150 mg/kg dose 3 and 4 h after treatment. The 2 h value with 37.5 mg/kg and the 3 h value at 75 mg/kg were also significantly different from the control value, $P<0.05$ and $P<0.01$ respectively. Hence the magnitude and duration of the hypoglycaemic effect are dose-dependent. The positive correlation between the degree and time of

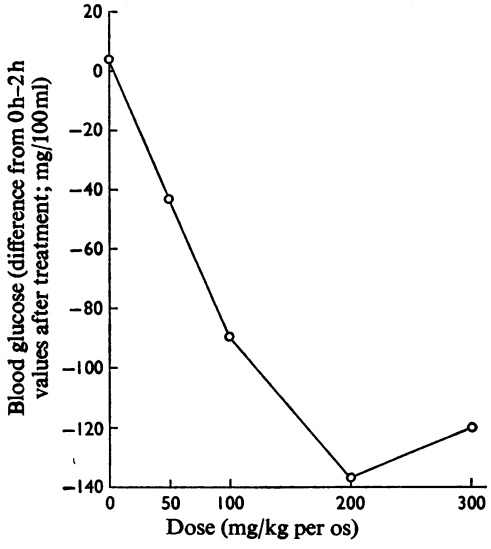

Fig. 3. *Hypoglycaemic effect of 3-mercaptopicolinic acid in alloxan-diabetic rats*

Alloxan-diabetic rats were given 3-mercaptopicolinic acid orally at doses of 50, 100, 200 and 300mg/kg suspended in 0.5% (w/v) tragacanth. Each point represents the mean tail-vein blood glucose value of nine rats obtained 2h after treatment.

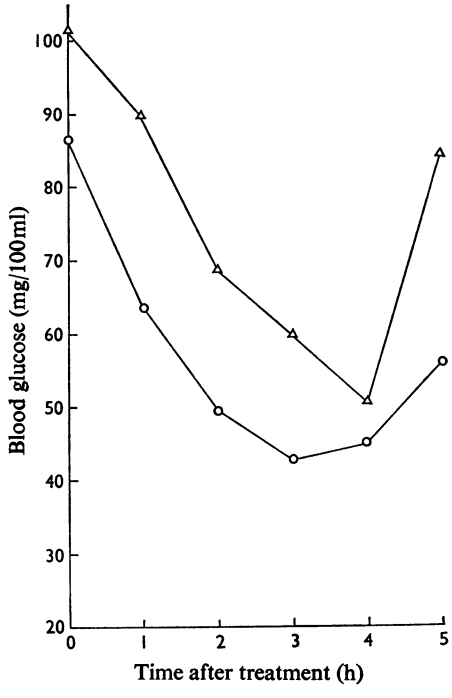

Fig. 4. *Hypoglycaemic effect of 3-mercaptopicolinic acid in starved mice and guinea pigs*

Mice (○) were starved for 19h and given 3-mercaptopicolinic acid at an oral dose of 150mg/kg in 0.5% (w/v) tragacanth. Repeated tail-vein blood samples were obtained from each mouse at the indicated times. Each point represents the mean for twelve mice. Guinea pigs (△) were starved for 48h and given 3-mercaptopicolinic acid at an oral dose of 125mg/kg in 0.5% (w/v) tragacanth. Each point represents the mean for ten killed animals.

greatest hypoglycaemia and the dose has consistently been observed in a number of experiments performed in rats starved for 18 or 48h after oral or intraperitoneal administration of 3-mercaptopicolinic acid.

In two experiments in 4h-post-absorptive rats, the oral administration of 3-mercaptopicolinic acid at a dose of 150mg/kg failed to elicit a significant hypoglycaemic response.

In 12h-starved acutely alloxan-diabetic rats, a significant ($P<0.01$) dose-related hypoglycaemic effect was observed with oral doses up to 200mg/kg at 2h after treatment (Fig. 3). Increasing the dose to 300mg/kg did not elicit a further decrease in the blood glucose. A similar plateau effect has been observed in the 48h-starved normal rat.

As well as in the rat, hypoglycaemic activity has been demonstrated in the 48h-starved guinea pig and the 19h-starved mouse. In the starved guinea pig, an oral dose of 125mg/kg significantly ($P<0.01$) lowered blood glucose at 2–5h after treatment (Fig. 4). The maximum effect was obtained at 4h, when the blood glucose had decreased to 51mg/100ml from a pretreatment value of 102mg/100ml. During the following hour, the blood glucose increased to a value of 84mg/100ml, which was still significantly ($P<0.05$) below the pretreatment value.

In starved mice, an oral dose of 150mg/kg significantly lowered ($P<0.001$) blood glucose at 1–5h (Fig. 4). At 3h after treatment, the blood glucose had decreased from a pretreatment value of 86mg/100ml to 43mg/100ml. In the subsequent 2h, the mean blood glucose value increased to 56mg/100ml; however, this value was still significantly ($P<0.001$) lower than the pretreatment value.

Effect on blood lactate

Since lactate is a significant precursor for gluconeogenesis in the starved rat (Ross *et al.*, 1967), its concentration in blood was determined in rats at various times after the administration of 3-mercaptopicolinic acid, and the values were correlated with blood glucose values. The results in Fig. 5 readily show a positive correlation between an increase in blood lactate and a decrease in blood glucose. Although not shown

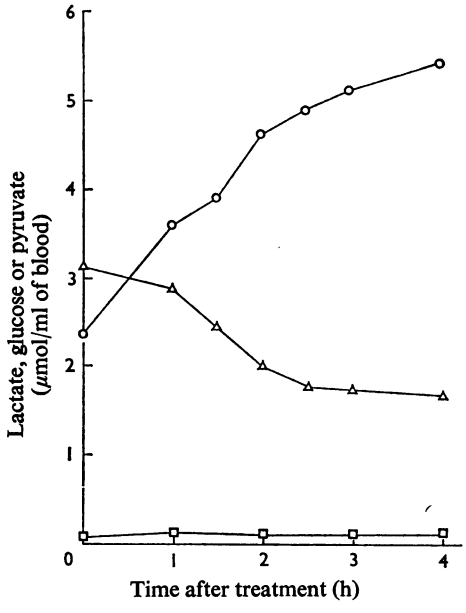

Fig. 5. *Effect of* 3-*mercaptopicolinic acid on blood lactate, pyruvate and glucose*

Rats starved for 48h were given 3-mercaptopicolinic acid orally at a dose of 125mg/kg suspended in 0.5% (w/v) tragacanth. Each rat was bled repeatedly from the tail vein at the indicated times for the determination of blood lactate (○), glucose (△) and pyruvate (□). Each point represents the mean values from twelve rats.

in Fig. 5, a group of eight control rats which were administered the vehicle were also repeatedly bled from the tail vein during the 4h period. At no time during the experiment did their blood lactate or pyruvate values show a significant deviation from the zero-time value, thus supporting the contention that the increase in blood lactate in the treated group was a result of inhibiting gluconeogenesis by 3-mercaptopicolinic acid. The lack of an effect on blood pyruvate resulted in significant increases in the [lactate]/[pyruvate] ratio from a zero-time value of 25 to 48 at 4h. Except for the 1h sample, the increment in μmol of blood lactate was twice the decrement in μmol of blood glucose. Since 2mol of lactate is required for each 1 mol of glucose synthesized, it indicates that the increase in blood lactate was due to decreased glucose synthesis. This assumption is valid only if, under the conditions of the experiment, the lactate that accumulated was distributed in a space equal to that of glucose.

Lactate oxidation

During the first and second 30min collection periods, the specific radioactivity of the expired CO_2 was significantly lower in the 3-mercaptopicolinic acid-treated rats (Table 3). However, the total CO_2 expired was slightly but significantly greater in the 3-mercaptopicolinic acid-treated rats during the second and third 30min collection periods, as was the total CO_2 produced in 90min. Hence the total amount of the tracer oxidized was not significantly different in control and treated rats. The data indicate that the trace dose of [^{14}C]lactate administered to 3-mercaptopicolinic acid-treated rats was diluted in a larger lactate pool, resulting in a decreased specific radioactivity of the expired CO_2. The data further indicate that the increase in blood lactate after administration of 3-mercaptopicolinic acid is not due to an inhibition of the oxidative process, since the amount of tracer oxidized was similar in control and treated rats.

Discussion

Numerous compounds have been shown to be inhibitors of gluconeogenesis and, as a consequence, induce hypoglycaemia under specific conditions (Haeckel, 1972; Lardy, 1969). The mechanisms by which gluconeogenesis is inhibited are as varied as the number of inhibitors.

The kidney and liver are the two major organs within the mammalian organism capable of gluconeogenesis. The kidney is of particular importance in studying gluconeogenesis *in vitro*, because it has a glycogen content of less than 0.01% (Krebs *et al.*, 1963; Krebs, 1963), thus negating the inhibition of glycogenolysis as a possible mechanism of decreased glucose production. Further, in the kidney the product of gluconeogenesis is glucose, not glycogen, decreasing the possibility of enhanced glycogenesis being responsible for the decrease in glucose liberated into the incubation medium. In the present experiments, 3-mercaptopicolinic acid exhibited a dose–response relationship in its ability to decrease glucose production from lactate by kidney-cortex slices.

In the control perfused livers, the highest rate of glucose production was from fructose, followed by pyruvate, lactate and alanine. This order of substrate preference is essentially the same as that published by Ross *et al.* (1967). Lactate, pyruvate and alanine were initially used as substrates in the perfused liver because the formation of glucose from them requires the participation of pyruvate carboxylase, phosphoenolpyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase, the four specific gluconeogenic enzymes. In contrast, fructose and dihydroxyacetone enter the gluconeogenic pathway at the level of dihydroxyacetone phosphate and glyceraldehyde phosphate, a point of entry requiring only the participation of fructose 1,6-diphosphatase

Table 3. *Effect of 3-mercaptopicolinic acid on oxidation of L-[3-^{14}C]lactate*

Values represent the mean ± S.D. for five rats. * and ** indicate statistical significance, $P < 0.05$ and $P < 0.01$, between control and 3-mercaptopicolinic acid-treated rats.

Treatment	Collection period (min)	CO_2 0–30 (d.p.m./mmol)	CO_2 0–30 (mmol)	CO_2 30–60 (d.p.m./mmol)	CO_2 30–60 (mmol)	60–90 (d.p.m./mmol)	60–90 (mmol)	Totals per 90min $10^{-5} \times {}^{14}CO_2$ (d.p.m.)	Totals per 90min CO_2 (mmol)
Controls (0.5% tragacanth, 4ml/kg per os)		72000 ±8530	5.1 ±0.8	106000 ±15200	4.2 ±0.4	85500 ±20200	4.1 ±0.5	11.6±1.9	13.4±1.3
+3-Mercaptopicolinic acid (125mg/kg, in 4 ml per os)		44600** ±11300	5.3 ±0.4	80700* ±11000	5.2* ±0.4	82200 ±6440	5.0* ±0.6	10.8±1.9	15.5±1.4*

and glucose 6-phosphatase. The ability of 3-mercaptopicolinic acid to inhibit glucose production from lactate, pyruvate and alanine indicates that the site of inhibition was not lactate dehydrogenase or alanine transaminase. At 60min of perfusion, essentially the same degree of inhibition was obtained with lactate, pyruvate and alanine, i.e. 47, 43 and 59% respectively. The inability of 3-mercaptopicolinic acid to inhibit glucose formation from fructose or dihydroxyacetone indicates that the site of inhibition was probably at pyruvate carboxylase or phosphoenolpyruvate carboxylase. A crossover plot, calculated from metabolite concentrations in freeze-clamped perfused livers, indicates phosphoenolpyruvate carboxylase as a possible site of inhibition (M. Goodman & G. F. Cahill, personal communication).

The inhibition of gluconeogenesis by 3-mercaptopicolinic acid when lactate, pyruvate or alanine served as substrate, was remarkable in its lack of effect on the other parameters measured. Comparing control and treated livers, there was no demonstrable effect on lactate, pyruvate or the [lactate]/[pyruvate] ratios in the medium, thus indicating the ability to maintain substrate utilization and a proper cytosol redox state under the influence of the inhibitor in the perfused liver.

When alanine served as substrate, the production of lactate and pyruvate was much less than for the other substrates used. However, in alanine-perfused livers, the presence of the inhibitor resulted in an approximate doubling of the lactate and pyruvate concentrations, with no effect on the [lactate]/[pyruvate] ratio.

In livers perfused with alanine, urea production was naturally greater than with the other substrates used. However, regardless of the substrate used, the presence of the inhibitor had no adverse effect on urea production. The lack of effect on urea production, which requires ATP and the maintenance of normal O_2 consumption, indicates that oxidative metabolism and oxidative phosphorylation were unaffected.

Since hepatic glycogen concentrations are unaffected, the decrease in the rate of glucose released by the perfused liver under the influence of the inhibitor was not a result of increased glycogenesis.

It is difficult to ascertain that the increase in blood lactate after administration of 3-mercaptopicolinic acid was due solely to the inhibition of gluconeogenesis. If the lactate accumulating in the blood of the treated group was in equilibrium with the total lactate space, which is approx. 60% of the body weight (Forbath *et al.*, 1967), then the increment in blood lactate was twice that which can be accounted for by the decrement in blood glucose, assuming a total space of 30% of body weight (Friedmann *et al.*, 1967). However, equilibration of exogenous lactate in the lactate space requires more than 4h in human beings (Kreisberg *et al.*, 1971) and 40min in abdominally

eviscerated dogs (Alpert, 1965). Lactate gradients are reported to exist between tissues and blood, indicating that lactate distribution in body water may be uneven (Kreisberg, 1972). In the present experiment, the blood lactate was increasing from endogenous sources, and the concentrations had not reached a plateau, indicating that the lactate pool was not equilibrated, and thus did not reflect the concentration of the total lactate space. Hence the accumulation of blood lactate may have reflected that arising from the inhibition of gluconeogenesis.

The inability to detect a decrease in the oxidation of a trace dose of [^{14}C]lactate in 3-mercaptopicolinic acid-treated rats indicated that the increase in blood lactate was not due to a decreased rate of oxidation.

The fact that a hypoglycaemic effect was only demonstrable in starved or diabetic animals is additional evidence that 3-mercaptopicolinic acid is an inhibitor of gluconeogenesis, since the process operates minimally in the post-absorptive state.

Quinolinic acid has been reported to be an inhibitor of gluconeogenesis (Veneziale *et al.*, 1967). However, quinolinic acid is a less effective inhibitor since, at a concentration of 1.2mM, it was only partially effective in inhibiting gluconeogenesis in perfused rat livers (Veneziale *et al.*, 1967). We obtained only a 27% inhibition of glucose production from lactate in the perfused liver with quinolinic acid at 2.3mM, in contrast with a 60% inhibition with 3-mercaptopicolinic acid at 0.1mM. Further, a hypoglycaemic effect was unattainable in the 48h-starved rat with an oral or intraperitoneal dose of 668mg/kg (4mmol/kg) of quinolinic acid. Experiments are designed to determine the mechanism of inhibition by 3-mercaptopicolinic acid.

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