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EFFECTS OF DERIVATIVES OF INDOLE CARBOXYLIC ACIDS ON CARBOHYDRATE METABOLISM OF THE RAT

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Abstract—Several indole derivatives were characterized on their effects on carbohydrate metabolism in the rat and compared in their insulin-like effects on hepatic gluconeogenesis and glucose consumption in muscle with 5-*O*-methoxyindole-2-carboxylic acid (MICA) and with indole-3-butyric acid. The substances depressed blood glucose in the fasted adrenalectomized rat at an oral dose of 50–250 mg/kg. Glucose consumption in an isolated muscle preparation was altered only a small amount. In some cases glycogen content of muscle was reduced at a millimolar concentration. All compounds revealed strong inhibition of glucose production from pyruvate in isolated liver slices at a concentration of 10^{-4} M. By chemical modification of the indole structure strong inhibitors of gluconeogenesis are obtained. It was not possible to find substances which show a high stimulation of glucose uptake and low activity in inhibiting gluconeogenesis.

MIRSKY[1,2] and Veneziale *et al.*[3] reported the effects of tryptophan and some of its metabolites on gluconeogenesis in rat liver. 5-Methoxyindole-2-carboxylic acid (MICA), a compound of structural similarity with tryptophan, which was first described by Bauman *et al.*,[4,5] also showed considerable inhibition hepatic gluconeogenesis.[6–8] Another group of compounds with related structure, i.e. indole-3-carboxylic acids, exhibited insulin-like properties on glucose metabolism; a stimulatory effect on glucose uptake could be demonstrated in the isolated hemidiaphragm of the rat.[9]

The purpose of the present paper was to investigate whether a differentiation between inhibition of hepatic gluconeogenesis and stimulation of muscle glucose uptake might be possible. Several new indole derivatives have been synthesized and tested in rats for their ability to lower blood glucose, to stimulate glucose consumption in an isolated muscle preparation and to inhibit glucose production from pyruvate in liver slices. The influence on $^{14}CO_2$-production from [^{14}C]glucose *in vivo* will be reported on in a second communication.†

MATERIAL AND METHODS

Indole carboxylic acids and tetrahydrocarbazole carboxylic acids were synthesized by Dr. H. Biere in the Department Arzneimittelchemie, Schering AG, Berlin. Details of the synthesis will be published elsewhere.‡ The structure of the compounds is shown

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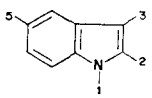
† Manuscript in preparation.
‡ H. Biere and E. Schröder, in preparation.

in Table 1. 5-Methoxyindole-2-carboxylic acid (MICA) was purchased from Fluka AG, Buchs, Switzerland, indole-3-butyric acid from E. Merck AG, Darmstadt, crystalline bovine insulin from Farbwerke Hoechst AG, Frankfurt/M. Test kits for the automated glucose determination were obtained from Boehringer GmbH, Mannheim. All other reagents were of analytical grade.

TABLE 1. STRUCTURE OF VARIOUS INDOLE CARBOXYLIC ACIDS AND TETRAHYDROCARBAZOLE CARBOXYLIC ACIDS



	1	2	3	5
5-Methoxy-indole-2-carboxylic acid	H	$-COOH$	H	$-OCH_3$
Indole-3-butyric acid	H	H	$-(CH_2)_3COOH$	H
I	$-CH_3$	H	$-(CH_2)_3COOH$	H
II	H	$-CH_3$	$-(CH_2)_3COOH$	H
III	$-(CH_2)_3COOH$	$-CH_3$	H	H
IV	H	$-CH(COOH)-CH_2-CH_2-CH_2-$		$-OCH_3$
V	H	$-CH(COOH)-CH_2-CH_2-CH_2-$		F
VI	H	$-CH(COOH)-CH_2-CH_2-CH_2-$		H

I 4-(1-Methyl-3-indolyl)-butyric acid,
II 4-(2-Methyl-3-indolyl)-butyric acid,
III 2-Methyl-l-indolyl-butyric acid,
IV 1-Carboxy-6-methoxy-1,2,3,4-tetrahydrocarbazole,
V 1-Carboxy-6-fluoro-1,2,3,4-tetrahydrocarbazole,
VI 1-Carboxy-1,2,3,4-tetrahydrocarbazole.

Glucose determination in adrenalectomized rats. Female Wistar rats (100–120 g body wt) were used in the experiments 3, 4 or 5 days after adrenalectomy. It has previously been established that glucose concentrations in blood are comparable 3, 4 and 5 days after surgery. Blood of adrenalectomized rats, fasted for 24 hr, was withdrawn from the orbital vein for the determination of glucose. Blood samples were taken before the application of the compounds and 90 and 180 min after the oral administration of the indole derivatives. The compounds were given as sodium salts or as aqueous microsuspensions by gavage. Glucose was determined using glucose oxidase[10] in the autoanalyzer Braun-Systematic. The glucose concentrations after application of the test compounds were expressed as per cent of

the initial value and were statistically compared with a control group given saline using the parameter free U-test.[11] The number of animals in each group was six.

Glucose uptake in the isolated soleus muscle. The procedure of Chaudry and Gould[12] was followed for the preparation and incubation of the tissue. Muscles of fasted male Wistar rats (100–120 g body wt), each weighing approximately 40 mg, were used in the experiments. The incubation medium consisted of 2 ml of Krebs–Henseleit bicarbonate buffer (pH 7·4), containing 10^{-2} M glucose and 10 mg/ml bovine serum albumin. Each incubation flask contained either two left or two right muscles with or without one of the indole derivatives. Thus each set of experiments had its own control. The concentration of the substances to be tested was 10^{-3} and 10^{-4} M. Incubations were carried out in a metabolic shaker for 1 hr at 37°. After the incubation aliquots of the medium were withdrawn, deproteinized with uranylacetate and the concentration of glucose determined automatically with glucose oxidase. As the weight of the muscles in both groups was approximately equal, glucose consumption is expressed in micrograms/incubation flask.

Glycogen determination in muscle tissue. Glycogen was determined using the method of Balzer and Palm.[13] At the end of the incubation both muscles were rinsed in buffer, slightly blotted on filter paper and transferred to a test tube containing 1 ml 30% KOH. After digestion of the tissue, glycogen was precipitated with ethanol, hydrolyzed with 1 N HCl and glucose was determined enzymatically. Glycogen content is expressed in glucose-equivalents/100 mg tissue wet wt. For statistical evaluation Wilcoxon's test for paired data was employed.[14]

Gluconeogenesis from pyruvate in liver slices. Approximately 150 mg of liver slices of fasted female Wistar rats (160–180 g) were incubated in 4 ml Krebs–Ringer bicarbonate buffer, containing 10^{-2} M sodium pyruvate. Glucose formation was measured in aliquots taken at different time intervals from the medium. Preliminary experiments without the addition of pyruvate have shown that the synthesis of glucose from endogenous substrates was small.* After a preincubation period of 105 min, which allowed the calculation of the normal velocity of glucose formation, either saline, MICA or one of the indole derivatives were added to the medium as sodium salts to yield a final concentration of 10^{-3} and 10^{-4} M, respectively. After an additional 75 min further glucose was determined. The inhibitory effect of a compound on gluconeogenesis was calculated from the change in the rate of glucose synthesis and expressed in per cent of the pre-treatment period. An inhibition of more than 100 per cent is explained by excessive glucose consumption from the medium. MICA was routinely used as a control in all experiments. The results are listed as medians and statistically evaluated employing the U-test.[11]

RESULTS AND DISCUSSION

The blood sugar lowering effect of several substituted indole carboxylic acids and tetrahydrocarbazole carboxylic acids is demonstrated in Table 1. Blood glucose depression in fasted adrenalectomized rats is expressed in per cent of the initial value after oral administration of the compounds. Compound I and II are approximately equally active as indole-3-butyric acid. The minimal dose of indole-3-butyric acid needed to give a significant blood glucose depression is 250 mg/kg. A significant effect on blood glucose in the adrenalectomized rat is already achieved with 50 mg/kg

* Unpublished observations.

TABLE 2. BLOOD GLUCOSE IN FASTED ADRENALECTOMIZED RATS AS PER CENT OF THE INITIAL VALUE AFTER ORAL ADMINISTRATION OF INDOLE CARBOXYLIC ACIDS AND TETRAHYDROCARBAZOLE CARBOXYLIC ACIDS

Compound	dose p.o. (mg/kg)	Blood glucose in % of the initial value after 90 and 180 min	
5-Methoxy-indole-2- carboxylic acid (MICA)	50	57·0*	60·0*
Indole-3-carboxylic acid	250	61·2* (n = 108)†	53·0
I	250	66·0*	53·5*
II	250	66·5*	55·5*
III	50	81·0*	74·5
IV	50	76·0*	74·0*
V	50	59·5*	67·5*
VI	50	71·5*	69·5*

* Significantly different from controls (n = 6).
† Median of 18 experiments with 6 animals each.
Each value represents the median of six animals.
For experimental details see Material and Methods.

with compounds III, IV, V and VI. Hence, these compounds lie within the dose range, in which 5-methoxy-indole-2-carboxylic acid (MICA) is effective.

Glucose consumption in the isolated soleus muscle of the rat is stimulated *in vitro* by both indole-3-butyric acid and MICA in millimolar concentrations (Table 3). This stimulatory effect of MICA on glucose uptake is not seen in the isolated hemidiaphragm.[15]* *In vivo*, glucose tolerance and $^{14}CO_2$-production from [^{14}C]glucose was shown to be reduced after application of MICA.[16] Increased glucose consumption is also observed with compound II and IV, however, glucose concentration during incubation was higher in these experiments. None of the other compounds is effective at 10^{-3} and 10^{-4} M, respectively. The glycogen content in muscle is only increased with 1 and 10 m units/ml insulin and with compound VI at a concentration of 10^{-4} M. This latter effect seems to be accidental. In contrast to insulin, compounds I, II and IV even cause a reduction in glycogen content.

The production of glucose from pyruvate in rat liver slices is inhibited by indole-3-butyric acid at a concentration of 10^{-3} M, and by MICA at 10^{-4} M (Table 3). All other indole derivatives show marked inhibition of hepatic gluconeogenesis at 10^{-4} M. Thus, the compounds tested are obviously more powerful inhibitors in this test system than indole-3-butyric acid.

From our results and from data in the literature evidence has accumulated that MICA and indole-3-butyric acid exert several effects on carbohydrate metabolism in the rat: (1) Blood glucose is depressed in the normal and in the adrenalectomized animal. As the substance also acts in alloxanized animals,[15] a β-cytotropic effect can be excluded. (2) The formation of glucose from pyruvate or alanine is inhibited in the perfused liver as well as in liver slices. (3) *In vivo*, MICA mobilizes glycogen stores,

* Unpublished observations.

TABLE 3. GLUCOSE CONSUMPTION AND GLYCOGEN CONTENT IN SOLEUS MUSCLE AFTER 1 HR INCUBATION WITH SEVERAL INDOLE CARBOXYLIC ACIDS AND TETRAHYDROCARBAZOLE CARBOXYLIC ACIDS (MEDIAN, n = 7)

Compound	Glucose consumption (μg/flask)			Glycogen content (μg/100 mg wet wt)		
	Control	10^{-3} M	10^{-4} M	Control	10^{-3} M	10^{-4} M
5-Methoxy-indole-2-carboxylic acid (MICA)	144 220	214†	332	29·1 30·3	27·6	45·5
Indole-3-butyric acid	358 360	380*	374	38·4 43·8	40·8	50·2
I	216 252	252	256	50·4 58·8	37·2†	55·4
II	400‡ 740‡	620*	620	41·6 74·0	35·7*	50·5
III	284 310	300	252	62·0 86·2	52·2	75·3
IV	1052§ 474§	1230†	990	21·0 15·1	14·4*	16·1
V	334 (n = 15) 124	328	70	22·6 (n = 15) 22·2	21·4	25·0
VI	260 266	328	296	36·5 25·4	38·2	30·2*
Insulin, 1 mU/ml	336	413*		36·4	62·8*	
Insulin, 10 mU/ml	254		263	23·0		49·3*

* $P < 0.05$.
† $P < 0.02$.
‡ Glucose concentration in this experiment was 500 mg%.
§ Glucose concentration in this experiment was 300 mg%.
For experimental details see Material and Methods.

because liver glycogen decreases despite high glucose concentrations evoked by glucose infusion.[5] (4) In the isolated soleus muscle glucose consumption is only stimulated at high concentrations of MICA. (5) Lipolysis in adipose tissue is not affected by MICA (unpublished results), however, after the administration of the compound *in vivo* the levels of free fatty acids, pyruvate and lactate increase in serum. Ketone bodies decrease and pyruvate, lactate, phosphoenolpyruvate, 2- and 3-phosphoglycerate accumulate in the liver.[7]

It therefore appears that the blood sugar lowering effect of MICA can be attributed primarily to an inhibition of glucose production in the liver. Lardy *et al.*[7,8] as well as Bauman *et al.*[4,5] have contributed to the elucidation of the mode of action of MICA. They have demonstrated in liver slices that the carboxylation of pyruvate to oxaloacetate and the oxidative decarboxylation of pyruvate and α-ketoglutarate are inhibited

by MICA. Phosphoenolpyruvate carboxykinase, one of the key enzymes in gluconeogenesis, is not influenced. The primary site of MICA-action appears to be on the level of mitochondrial α-keto-acid oxidation. In addition to the inhibition of these steps, the formation of acetoacetate from palmitoylcarnitin via acetyl-CoA appears to be affected. This could, at least partially, explain the inhibition of free fatty acid oxidation and the subsequent increase in free fatty acids in serum. Palmitoyl-carnitin, in higher concentrations, as well as octanoate and 2,4-dinitrophenol, can reverse the inhibition of gluconeogenesis in the liver. It has been demonstrated by Reed and Lardy[8] that MICA can be concentrated in mitochondria approximately 30-fold and that the penetration of the indole derivative is inhibited by octanoate and 2,4-dinitrophenol. Intramitochondrially, lipoyldehydrogenase, an essential enzyme in the oxidative decarboxylation of pyruvate, seems to be inhibited, an effect which could be shown *in vitro*.

As the mitochondrial carboxylation of pyruvate to C_4-dicarboxylic acids which are responsible for the transfer of hydrogen from mitochondria into the cytosol, is depressed by MICA, the levels of malate, oxaloacetate and NADH in the cytoplasm in consequence will decrease. Thus, in the liver, the NADH-dependent conversion of 3-phosphoglycerate to glyceraldehyde-3-phosphate and hence the formation of glucose is blocked. A schematic description of the main points of interference of MICA in carbohydrate metabolism is given in Fig. 1.

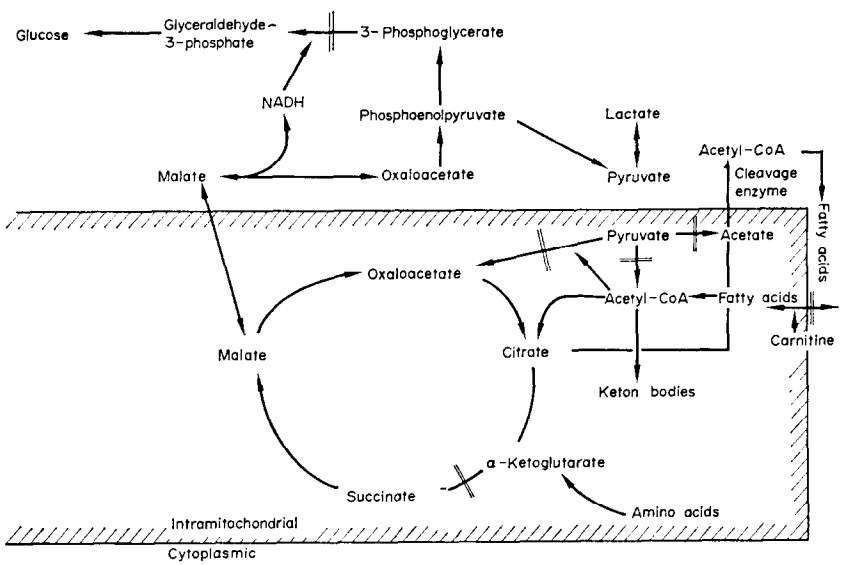


FIG. 1. Main points of interference of MICA in carbohydrate metabolism.

Much less data are available on the mode of action of indole-3-butyric acid. Blood glucose is depressed after oral application in the intact mouse, the adrenalectomized and intact rat. The effect in the intact rat is not reproducible with certainty. After oral administration of indole-3-butyric acid, glycogen in the liver of the rat is decreased. Free fatty acids in serum increase, although less pronounced than after the application of MICA. The formation of glucose in liver slices of the rat is inhibited at a tenfold

higher concentration than MICA. Both indole-3-butyric acid and MICA, enhance the consumption of glucose in the isolated soleus muscle (Table 3). In the isolated diaphragm, however, only indole-3-butyric acid has been found to increase glucose uptake[9] (unpublished results). *In vivo*, this compound causes a small increase in $^{14}CO_2$-production from [U-^{14}C]glucose.

TABLE 4. INHIBITION, IN PER CENT, OF GLUCOSE FORMATION FROM PYRUVATE IN LIVER SLICES OF THE RAT

Compound	Glucose production in liver slices (% Inhibition)			
	Saline	10^{-3} M	10^{-4} M	Standard (MICA 10^{-4} M)
5-Methoxyindole-2-carboxylic acid (MICA)	23	114	113	
Indole-3-butyric acid	21·5	118·9	10·8	97·9
I	36·6	109·5	71·7	111·7
II	34·0	181·8	64·1	88·4
III	31·8	100·6	87·8	91·2
IV	22·7	160·3	41·3	87·8
V	42·8	65·7	69·9	96·5
VI	29·1	122·6	67·5	107·8

All values (n = 4) are statistically different (U-test) from the controls except indole-3-butyric acid, 10^{-4} M.
For experimental details see Material and Methods.

The newly synthesized indole carboxylic acids and tetrahydrocarbazole carboxylic acids show effects comparable to those of MICA and indole-3-butyric acid. All compounds exhibit blood sugar lowering activity. The formation of glucose from pyruvate in liver slices was almost completely depressed by concentrations as low as 10^{-4} M. Most likely, the compounds also inhibit gluconeogenesis in the liver of the rat.

In the isolated muscle preparation insulin stimulates both glucose consumption and glycogen deposition, the latter effect being more pronounced than the first. In our experiments, one substituted indole carboxylic acid (II) and one tetrahydrocarbazole carboxylic acid (IV) increased glucose consumption. This effect was, however, combined with a significant reduction in glycogen content. A similar decrease was exhibited with the indole derivate I. It must, therefore, be concluded that in muscle the indole derivatives do not show increased insulin-like activity compared to indole-3-butyric acid and MICA.

In summary, the present experiments do not support the concept that a differentiation, by chemical modification of the indole structure, between inhibition of gluconeogenesis and stimulation of glucose consumption, might be possible.

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