Biochemical Pharmacology, Vol. 25, pp. 2285–2289. Pergamon Press, 1976. Printed in Great Britain.

INHIBITION OF KIDNEY CORTEX GLUCONEOGENESIS BY ADRENOCHROME AND INDOLE 2-CARBOXYLIC ACID

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(*Received* 2 *March* 1976; *accepted* 28 *April* 1976)

Abstract—Gluconeogenesis in renal cortex slices was inhibited by adrenochrome and indole 2-carboxylic acid, at concentrations in the range 3–50 μg/ml. Inhibition was extensive with pyruvate as substrate, and with glutamine in the case of adrenochrome, but was less marked from glycerol and fructose. Butyrate reversed the inhibition by indole 2-carboxylic acid, but not that by adrenochrome. Both drug effects were reversible on washing the tissue. Less potent effects on gluconeogenesis were exerted by quinolinate in kidney (30 per cent inhibition at 50 μg/ml) and adrenochrome in perfused liver (no effect at 25 μg/ml). These actions are discussed in regard to their significance, and mechanism.

The quest for inhibitors of gluconeogenesis is important for three reasons. Firstly, study of such agents might lead to advances in the therapy of diabetes. Secondly, inhibitors can serve as useful tools in the study of biochemical mechanisms in tissues. Thirdly, where these inhibitors are naturally-occurring, their effects may reveal clues about normal control processes.

Among the most powerful inhibitors of gluconeogenesis are compounds of the type which includes tryptophan [1, 2] its metabolite quinolinate [3, 5] and an analogue mercaptopicolinic acid [6–8]. Tryptophan is an indole derivative, and other indole compounds can also cause potent inhibition of gluconeogenesis; indole carboxylic acids are among the most widely studied [9–13]. The effects of these agents are complex [8, 12–15], and their mechanism of action is not fully resolved, although pyruvate metabolism appears to be affected [9, 11–13, 15], and the monocyclic acids can inhibit the enzyme phosphoenolpyruvate carboxykinase in particular [3, 7, 8, 16, 17].

The above studies have usually concerned glucose synthesis by liver rather than kidney. Quinolinate, which has featured in discrete studies with kidney cortex [18, 19] is not particularly potent in its action on kidney, as is shown in the present paper. Methoxyindole 2-carboxylic acid has been reported to have less effect on substrate oxidation in kidney, compared to liver [11]. The results presented here extend understanding of the inhibition by indole derivatives in particular, of renal gluconeogenesis, in demonstrating potent effects of indole 2-carboxylic acid, and of adrenochrome (a naturally-occurring indole).

MATERIALS AND METHODS

Cortex slices were cut freehand, with a microtome blade and guide, from kidneys of male Sprague–Dawley rats, weighing about 200 g, and starved for 48 hr. They were incubated in 4 ml of Krebs–Ringer–bicarbonate saline, gassed with 5% carbon dioxide in oxygen. Indole 2-carboxylic acid was purchased from Sigma Ltd and dissolved in 0.15 M sodium bicarbonate. Adrenochrome and quinolinic acid (Sigma Ltd) were dissolved in water. Fresh solutions (1 mg/ml) were made each day, adjusted to pH 7.4 with HCl or NaOH, and kept in ice. Substrates (Analar) were from C. F. Boehringer or Sigma, Ltd.

Incubation was terminated with 0.4 ml 20% (w/v) perchloric acid. Glucose was analyzed by a glucose oxidase method [20]. Adrenochrome in standard samples reduced the observed extinctions, as can other indole compounds [21]. This phenomenon was not observed if glucose standard (internal) was added to acid-treated incubation media, and then measured. Nevertheless, such internal standards were employed for all assays in media which contained adrenochrome; two portions of the acidified medium were taken, and an extra known amount of glucose was added to one sample. Glucose was determined in both, to check that normal colour was obtained with added glucose. Indole 2-carboxylic acid did not affect the glucose assay. Pyruvate was measured enzymatically [22].

Experiments were designed to test variables on slices from the same rat(s), with appropriate controls; thus, several mean values for pyruvate-dependent gluconeogenesis are presented, each representing that obtained with control slices in particular experiments.

Livers were perfused with a bicarbonate-buffered saline containing bovine serum albumin and rat erythrocytes [23].

RESULTS

Characteristics of action of indoles on renal gluconeogenesis. Gluconeogenesis from pyruvate was inhibited by both indole derivatives, adrenochrome and indole 2-carboxylic acid (Fig. 1). The effect of indole 2-carboxylic acid was especially potent (range 2–10 μg/ml). The concentration-dependence of the action of adrenochrome on gluconeogenesis from pyruvate (Fig. 1) resembled that on gluconeogenesis from lactate or glutamine (details not shown). Quinolinate exerted a less potent inhibition (Fig. 1). Pyruvate uptake in the presence of indoles was also followed. Adrenochrome reduced pyruvate uptake, by an amount which corresponded approximately to the decline in glucose synthesis (Table 1). Indole 2-carboxylic acid had no effect on pyruvate uptake (results not shown).

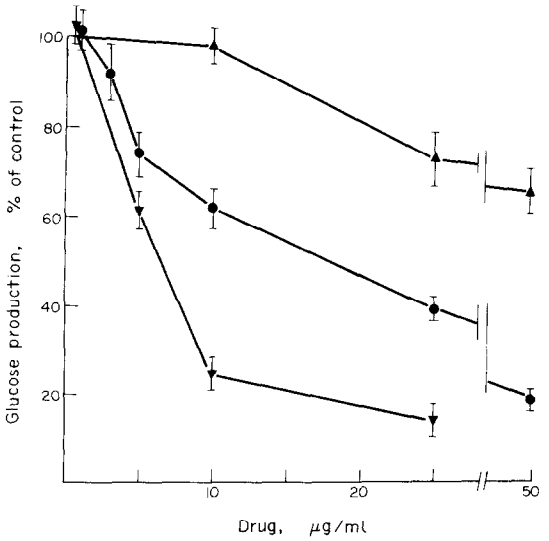

Fig. 1. Dependence on concentration of indole derivatives of inhibition of renal gluconeogenesis. Cortex slices were incubated as described in the text, with 10 mM sodium pyruvate, and glucose synthesis measured. The control rate (100% in the Fig.) was 258 ± 18 (12) μmole/hr/g dry wt. Drugs were: ▲, quinolinate; ●, adrenochrome; ▼, indole 2-carboxylic acid. Results are means of 3–5 measurements, and bars indicate the S.E.M.

Effects of indoles on glucose synthesis from precursors other than pyruvate were investigated. The inhibitory action of adrenochrome and indole 2-carboxylic acid was less marked when fructose or glycerol was the substrate (Table 2). In the presence of precursors which feed into the citrate cycle, a divergence of effect was revealed, i.e. adrenochrome inhibited gluconeogenesis from glutamine much more markedly than did indole 2-carboxylic acid (Table 2). Indeed the inhibition from glutamine was proportionally greater than that with any other substrate (Table 2).

Reversibility of indole effects. Interest in powerful inhibitory actions such as of Fig. 1. and Table 2 is greater if the effects are reversible, as this property separates "damage" effects from those which reflect reversible regulatory interactions with the cellular metabolic apparatus.

This aspect of indole action on gluconeogenesis was tested in two ways. First, the further addition of a highly oxidisable substrate, butyrate, was studied.

Table 1. Influence of adrenochrome on pyruvate uptake

Metabolic changes (μmole/g dry wt)	Control	Adrenochrome (25 μg/ml)
Glucose	246 ± 6 (4)	94 ± 1 (3)
Pyruvate	−1270 ± 250 (4)	−900 ± 75 (3)
Calculated changes due to adrenochrome		
Decrease in pyruvate uptake		370
Decrease in glucose synthesis		152
Pyruvate corresponding to glucose decrease		304

Kidney slices were incubated as described in the text, and pyruvate and glucose measured in initial and final medium samples. Results are means ± S.E.M. of the number of observations in parenthesis.

Table 2. Effect of indole derivatives on glucose synthesis from various substrates

Substrate (10 mM)	Glucose synthesis (μmole/hr/g dry wt) Control	Adrenochrome (10 μg/ml)	ICA (10 μg/ml)
Fructose	416 ± 47 (6)	405 ± 15 (3)	385 ± 30 (3)
Glycerol	91 ± 2 (6)	69 ± 4 (4)	
Glutamine	98 ± 4 (10)	30 ± 4 (5)	84 ± 4 (3)
Malate	445 ± 8 (3)	289 ± 15 (3)	
Lactate	219 ± 14 (3)	148 ± 3 (3)	
Pyruvate	258 ± 18 (12)	161 ± 10 (15)	95 ± 5 (4)

Kidney cortex slices from 48 hr-starved rats were incubated as described in the text, for 1 hr at 37°. Results are means ± S.E.M. from the number of flasks in parenthesis.
Abbreviations: ICA: indole-2 carboxylic acid.

This fuel reversed the action of indole 2-carboxylic acid on glucose synthesis from pyruvate (Table 3) but had relatively little effect on the inhibitory action of adrenochrome (Table 3).

Secondly, in view of the failure of butyrate on adrenochrome action, reversal was attempted with a washing procedure. The inhibitory effect was completely reversed after contact with the agent for 15 min (Table 4). Longer contact (30 and 45 min) produced effects which were not fully reversible (30–50% reversible, if rates in the period after drug wash-out are compared with control rates in the second period: Table 4). The action of indole 2-carboxylic acid was fully reversible after 30 min contact (Table 4). The data in Table 4 imply that the inhibitory action of adrenochrome was less marked in the shortest time period tested (15 min) compared to longer periods; such a trend, which could imply that an agent is acting indirectly by forming a derivative within the tissue, was not confirmed in separate experiments designed specificity to test this possibility (results not shown).

Action of adrenochrome on hepatic gluconeogenesis. It is well established that indole 2-carboxylic acids (especially the 5-methoxy-analogue) can inhibit glucose synthesis in the liver [9, 13]. However this aspect of adrenochrome action has not been studied. To tackle this question, the perfused liver preparation [23] was employed; no effect of adrenochrome (25 μg/ml, which exerted a near-maximal effect on kidney cortex; Fig. 1) was discerned (Fig. 2). Although erythrocytes and albumin were present in perfusate, the lack of action of adrenochrome was not a result of these factors, as was shown in separate perfusions in their absence (results not shown).

Table 3. Influence of butyrate on inhibition of glucose synthesis by indole derivatives

Substrate (10 mM)	Drug (μg/ml)	Glucose synthesis (μmole/hr/g dry wt) Control	Butyrate (10 mM)
Pyruvate	None	370 ± 11 (6)	431 ± 80 (6)
Pyruvate	Adrenochrome, (25)	137 ± 13 (3)	191 ± 6 (3)
Pyruvate	ICA, (10)	95 ± 4 (5)	428 ± 25 (3)
Glutamine	None	98 ± 4 (10)	110 ± 8 (3)
Glutamine	Adrenochrome, (25)	38 ± 5 (3)	30 ± 8 (3)

Gluconeogenesis was measured as in Table 1.

Table 4. Reversibility of indole effect on washing of slices

Drug	Duration of period (min)	Period 1 Drug	Period 1 Glucose formed	Period 2 Drug	Period 2 Glucose formed
Adrenochrome	15		82 ± 12 (3)		75 ± 12 (3)
(25 μg/ml):	15	+	60 ± 6 (3)	+	44 ± 2 (3)
	15	+	54 ± 9 (4)	−	87 ± 10 (4)
	30	−	160 ± 16 (4)	−	139 ± 13 (4)
	30	+	88 ± 9 (4)	+	91 ± 9 (4)
	30	+	77 ± 12 (4)	−	116 ± 18 (4)
	45	−	243 ± 8 (3)	−	187 ± 3 (3)
	45	+	122 ± 22 (3)	+	117 ± 15 (3)
	45	+	104 ± 9 (5)	−	142 ± 10 (5)
ICA (10 μg/ml):	30	−	158 ± 16 (3)	−	115 ± 8 (3)
	30	+	59 ± 19 (3)	+	69 (2)
	30	+	64 ± 8 (3)	−	124 ± 20 (3)

Kidney slices were incubated with pyruvate (10 mM) for two periods, of duration indicated. In some groups, drug was present in the first period, but not the second. Slices were blotted and inserted in new (pre-warmed) medium for the second period in all groups (even when the media were initially identical). Groups of three pairs of flasks (for each time value) always contained slices from kidneys from the same rat. Glucose formed, measured at the end of each period, is expressed as μmole per g dry slice, weighed at the end of the second period. Other details are in the text. Results are means ± S.E.M. of the number of observations in parenthesis.

DISCUSSION

Action of adrenochrome on kidney cortex. The inhibitory action of adrenochrome on gluconeogenesis in the kidney cortex is interesting for a variety of reasons. First, this effect was relatively potent (being exerted over the range 3–50 μg/ml). Also, the effect did not involve non-specific damage to the tissue, as it was reversible, and specific to glucogenic substrates which enter the pathway "below" triose-phosphate. Hence this finding adds a new kind of compound (quinonoid indole derivatives) to the list which can inhibit glucose synthesis, and which in general warrant follow-up in regard to possible usefulness in diabetes. The hypoglycaemic effect of adrenochrome itself *in vivo* is not great (for review see ref. [24]) but that of analogues might be greater.

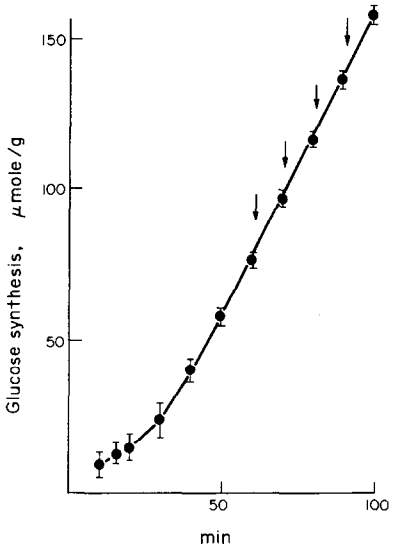

Fig. 2. Effect of adrenochrome on gluconeogenesis in the perfused liver. Livers from 48-hr starved rats were perfused with a mixture of lactate, glycerol and pyruvate, which was added as an initial dose, and then infused [23]. At four times, and at 10-min intervals (60–90 min after the start of perfusion) adrenochrome was added (in 50 μl) to a calculated concentration of 25 μg/ml. Transient vasoconstriction was observed each time (20% decrease in flow for 1–2 min). Results (means ± S.E.M.) are from three perfusions.

Also, the action of adrenochrome is more potent on kidney cortex, than on liver. This could have a variety of origins. One possibility is that adrenochrome is converted to active derivative(s) within the kidney; thus an oxidase which could catalyze such a change is present in kidney (albeit to a limited extent) but not in liver [25]. However, this is perhaps unlikely in view of the rapid action of adrenochrome (within 15 min). Such specificity of tissue response suggests that further study of the mechanisms of adrenochrome action would reveal aspects of gluconeogenesis which are specific to kidney.

Adrenochrome is a naturally-occurring derivative of adrenalin, which can easily be formed by spontaneous oxidation (for references see [26]). Therefore it is possible that it could contribute a feedback inhibitory component to the activation of gluconeogenesis in kidney cortex by catecholamines [27–29]. Conversely, the role of cellular "anti-oxidants" (such as ascorbic acid, glutathione) in preventing adrenalin oxidation, can be seen to be significant in cell function in the light of the powerful effect of adrenochrome. This conclusion is not vitiated by the likelihood that tissue adrenochrome concentrations do not attain levels employed in incubations, as local concentrations produced from adrenalin could be high in regions of high oxygen tension. As the action of adrenochrome was relatively reversible (by wash-out), transient effects of such compounds could occur in cells, which would not sustain irreversible damage. Also, some effects of added adrenalin (although not on gluconeogenesis) could be due to adrenochrome formation in tissues (e.g. [24, 30–32]). In general, the metabolic effects of adrenochrome have not been much studied, even during the long period of interest in its possible role in mental disease; reported effects in brain include those on glutamate decarboxylase [33, 34] and glycolysis [35].

Action of indole 2-carboxylic acid on kidney cortex. The inhibition of renal gluconeogenesis by indole 2-carboxylic acid (range 2–10 μg/ml) is among the most potent such effects yet reported for this kind of compound. Another equally potent action is that of mercaptopicolinate (e.g. 38% inhibition of gluconeogenesis from pyruvate at about 1.5 μg/ml.: ref. 7). In view of the sensitivity of these responses, the effects of these simple heterocylic carboxylic acids warrant further study, for the reasons given above (in regard to adrenochrome action).

Mechanism of effects of indole derivatives on kidney cortex. The question arises of the mechanisms of action of indole compounds on gluconeogenesis. The inhibitory effects of adrenochrome and indole 2-carboxylic acid are different in their nature, as shown by the differences in (i) reversibility with butyrate or by washout (ii) action with glutamine as substrate (iii) effect on pyruvate uptake. However, both indole deri-

vatives exerted relatively little action on gluconeogenesis from fructose or glycerol. This suggests that the inhibitory effects in the presence of other substrates are exerted at steps in the gluconeogenic pathway which precede the aldolase reaction. The most important such step is that catalyzed by phosphoenolpyruvate carboxykinase, which could therefore be a site of action of the indole compounds, as it is for quinolinate and mercaptopicolinate [3, 7, 8, 16, 17].

The inhibitory action of indole 2-carboxylic acids on gluconeogenesis has been studied in liver preparations. Major inhibitory sites of action appear to be at pyruvate carboxylase and dehydrogenase steps [11, 15] although further sites may exist [12, 13]. The reversibility of its inhibitory action by butyrate is compatible with an effect on pyruvate metabolism in kidney, as short-chain acyl-CoAs are powerful activators of pyruvate carboxylase [36]; this effect could reverse that of an agent which inhibited pyruvate carboxylase. The relative lack of effects of indole 2-carboxylic acid with glutamine as substrate and on pyruvate uptake are also in keeping with the view that indole carboxylic acids act relatively specifically on pyruvate metabolism, directing carbon preferentially away from glucose synthesis and perhaps from oxidation. Part of the explanation for its action could lie in a deficient provision of pyruvate for oxidation and ATP maintenance, which would be easily reversed by butyrate. In kidney, such possibilities remain to be investigated. There could be differences in mechanisms between tissues; thus the action of 5-methoxyindole 2-carboxylic acid in adipose tissue may not resemble that in liver [13].

The action of adrenochrome on renal gluconeogenesis may be more complex than that of indole 2-carboxylic acid, as shown by the potent inhibition of gluconeogenesis from glutamine as well as from pyruvate, and by the inhibition of pyruvate uptake. Yet the inhibition with malate as substrate was less marked. Thus adrenochrome may not greatly affect the steps of gluconeogenesis between oxalacetate and glucose, or for that matter the citrate cycle, as also indicated by the fact that pyruvate uptake was decreased by an amount approximately corresponding to the decline in glucose synthesis. This latter feature (not observed with indole 2-carboxylic acid) could indicate that pyruvate entry to cells was affected, but this is unlikely as similar inhibition would require to be invoked for other substrates, and all such substrates appear to enter kidney freely.

In view of its relative lack of effect on malate-dependent gluconeogenesis, the action of adrenochrome in the presence of glutamine may be specific to initial steps in glutamine metabolism. This possibility is reminiscent of a similar conclusion in regard to quinolinate action on kidney cortex [18]. Alternatively, since malate-dependent gluconeogenesis was inhibited to some extent, it could be that adrenochrome acts at the phosphoenol pyruvate carboxykinase reaction. This is the best single-site theory which can explain the actions of adrenochrome reported here. Then the relatively greater effect with glutamine (than with malate) could be explained, e.g., by a less effective maintenance of tissue oxaloacetate level by glutamine, so that inhibition at this step exerted a greater proportional effect. The occurrence of significant inhibition from malate excludes as sole site of action the possibility of effects on mitochondrial transport processes, as conversion of malate to glucose in rat kidney cortex is an extra-mitochondrial process. However, such effects (which can be very potent, eg. [37]) could contribute to the inhibition of gluconeogenesis from glutamine or pyruvate.

Although adrenochrome and indole 2-carboxylic acid are both indole derivatives, it is noteworthy that there are major differences in their action on gluconeogenesis. These must arise from the detailed differences in structure, of which the most significant is perhaps the quinone function on the ring of adrenochrome. If the quinone function has a role in adrenochrome action, then redox reactions would be expected to be implicated; phosphoenolpyruvate carboxykinase is vulnerable to such attack (as the active enzyme involves a Fe^{2+}-protein complex [3]), as are enzymes with vulnerable—SH groups; thus adrenochrome has been shown to react with such groups [38, 39].

Acknowledgements—We thank C. S. Harmon Esq. for carrying out the experiments with the perfused liver.

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