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THE EFFECTS OF MEPYRAPONE (SU 4885) AND SOME HYPERCHOLESTEROLAEMIC DRUGS ON HEPATIC STEROL AND FATTY ACID OXIDATION

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Abstract—The oxidation of several sterols by mouse liver mitochondria has been found to be inhibited by mepyrapone; 26-hydroxycholesterol, cholesterol oxidation and to a lesser extent, 7α-hydroxycholesterol oxidation were inhibited by mepyrapone, whereas 3β-hydroxycholest-5-en-26-oic acid oxidation was hardly affected. The mitochondrial oxidation of several fatty acids was unaffected by added mepyrapone even at relatively high concentrations of the drug. Three purified NAD- linked dehydrogenases, including 26-hydroxysterol dehydrogenase, were found to be selectively inhibited by mepyrapone when compared with an analogue, 3-acetylpyridine.

Several other drugs in the group which inhibit steroid hydroxylations in the adrenal cortex (SU series) also inhibited cholesterol oxidation but not fatty acid oxidation in the liver. Three drugs which inhibit cholesterolgenesis in the liver (SKF series) were found to inhibit sterol oxidation by liver mitochondria. These drugs differed from the SU series in their effects on fatty acid oxidation by both heart and liver mitochondria. The stimulation of fatty acid oxidation by SKF 525A appeared to mimic the effects of added DL-carnitine especially in heart mitochondria.

Whereas carnitine stimulated the mitochondria oxidation of 3β-hydroxycholest-5-en-26-oic acid, it was found that SKF 525A inhibited the latter's oxidation. From detailed studies comparing the effects of SKF 525A and carnitine, it was concluded that SKF 525A may antagonize carnitine at low concentrations of the latter. These effects were observed mainly with palmitate oxidation in heart mitochondria.

A NUMBER of drugs have been described which inhibit sterol and steroid metabolism notably adrenal corticoid inhibitors such as mepyrapone (SU 4885)[1] and inhibitors of cholesterolgenesis based on diethylaminoethanol such as triparanol (MER 29) and β-diethylaminoethyl 2,2-diphenylvalerate (SKF 525A)[2]. The chemical formulae of these drugs are depicted in Fig. 1.

This report describes the effects of these and related compounds on the oxidation of cholesterol and other substrates by liver enzymes. These correspond to various intermediates in the scheme of cholesterol oxidation (side chain degradation), outlined in Fig. 2.[3,4]

EXPERIMENTAL

Methods

Sources of supply for special materials were as follows: [26-^{14}C]-cholesterol,

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sodium [1-^{14}C]-octanoate, sodium [2-^{14}C]-propionate and [1-^{14}C]-palmitate—the Radiochemical Centre, Amersham, Bucks; Tween 20—Atlas Powder Co., Wilmington, Delaware, U.S.A.; SKF drugs were donated by Dr. W. Holmes (Smith, Klyne and

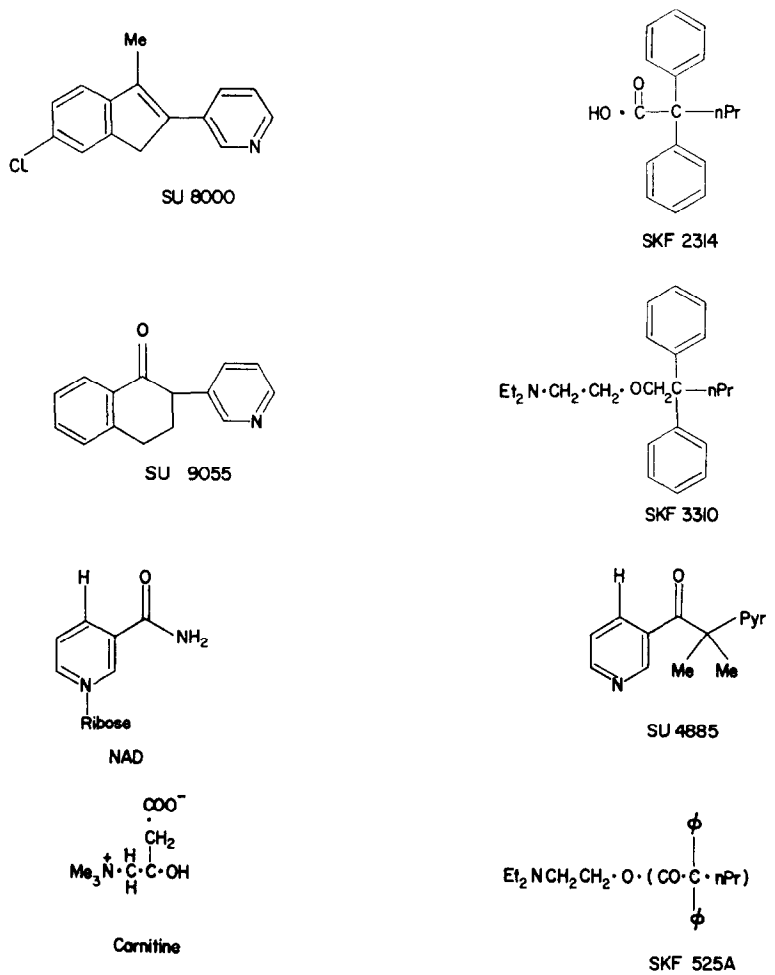

FIG. 1. Drugs which either inhibit cholesterogenesis (SKF series) or steroid hydroxylation (SU series) Structure comparison of SKF 525A with carnitine and SU 4885 with the pyridine moiety of NAD.

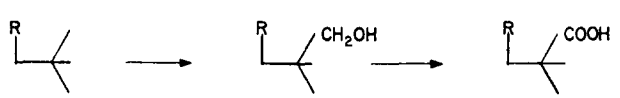

FIG. 2. Some intermediates in sterol side-chain oxidation.
R = C_{23} fragment.

French Laboratories, Philadelphia, U.S.A.); SU drugs by Dr. H. Sheppard (Ciba Laboratories, Summit, New Jersey, U.S.A.).

[26-^{14}C]-26-hydroxycholesterol, [26-^{14}C]-7α-hydroxy-cholesterol and [26-^{14}C]-3β-hydroxycholest-5-en-26-oic acid and [1-^{14}C]-2-methyloctanoate were synthesized as described by Dean and Whitehouse.[4,5]

Enzyme preparations were obtained from livers of male rats or female mice or from ox hearts[4,5] and incubated with ^{14}C-substrates as described elsewhere.[4]

RESULTS

Experiments with mepyrapone (SU 4885)

The effects of mepyrapone and 3-acetylpyridine on the oxidation to $^{14}CO_2$ of cholesterol and of sterols more polar than cholesterol, by liver mitochondria are summarized in Tables 1 and 2. Mitochondria fatty acid oxidation was also measured in the presence of these compounds to ascertain whether or not these drugs were non-specific inhibitors of mitochondrial oxidation.

TABLE 1. THE INHIBITION OF THE CHOLESTEROL OXIDASE SYSTEM AND OTHER ENZYMES BY (SU 4885)

Assay used	Percentage inhibition at drug concentration (mM) indicated				
	0	0·1	0·25	0·5	1·0
26-hydroxy-chol → $^{14}CO_2$	0	15	33	48	60
26-hydroxy-chol → Cholestenoate	0	ND	ND	80	80
7α-hydroxy-chol → $^{14}CO_2$	0	8	16	23	40
Cholestenoate → $^{14}CO_2$	0	0	−12	10	9
Cholesterol → $^{14}CO_2$	0	16	26	48	59
Propionate → $^{14}CO_2$	0	ND	ND	−6	ND
β-HO-Butyrate + NAD → NADH	0	0	ND	ND	30*
Ethanol + NAD → NADH			inhibits, see text†		

A negative value indicates stimulation compared with control; ND = not determined.

* D. H. Williamson, personal communication.

† M. Dickinson, personal communication.

TABLE 2. THE EFFECTS OF DRUGS WHICH INHIBIT EITHER CHOLESTEROL SYNTHESIS (SKF SERIES) OR STEROID HYDROXYLATION (SU SERIES) ON THE OXIDATION OF LIPID SUBSTRATES TO CO_2

Substrate	No drug	SU 4885	SKF 525A	SKF 2314	SKF 3301	3-Acetyl-Pyridine
(a) 2-Methyloctanoate	100	105	103	102	105	100
Octanoate	100	100	120	170	200	100
Cholestenoate	100	90	30	60	40	100
26-Hydroxycholesterol	100	—	—	—	—	100
(b) Cholestenoate	—	2·0	0·35	0·15	0·42	—
26-Hydroxycholesterol	—	0·9	0·4	0·45	0·12	—
7α-Hydroxycholesterol	—	1·1	0·75	—	—	—
Cholesterol	—	0·1	—	—	—	—

(a) Results given as percentages of control; drug concentrations 0·5 mM;

(b) Results given as concentration of drug (mM) to produce 50 per cent inhibition.

It was found that mepyrapone had no effect on $^{14}CO_2$ formation from the oxidation of the ^{14}C-fatty acids studied (octanoate, propionate and 2-methyloctanoate).

The oxidation to $^{14}CO_2$ of a steroid α-methyl carboxylic acid, cholestenoate (3β-hydroxycholest-5-en-26-oic acid), was not significantly inhibited by mepyrapone.

By contrast the oxidation of three sterols, namely cholesterol, 26-hydroxy-cholesterol and to a less extent 7α-hydroxycholesterol, was inhibited by mepyrapone. A partly purified preparation of the mitochondrial enzyme which oxidizes 26-hydroxycholesterol to cholestenoic acid in the presence of NAD, 26-hydroxysterol dehydrogenase,[4] was more sensitive to this drug than was the oxidation of 26-hydroxycholesterol by intact mitochondria (Table 1). The relative specificity of these effects of mepyrapone was indicated by the fact that 3-acetylpyridine (up to 0·5 mM) had no measurable effect on these enzymes.

It was found that at least two other NAD-linked dehydrogenases were also inhibited by mepyrapone. These are β-hydroxybutyrate dehydrogenase extracted from *Rhodopseudomonas spheroides* and purified horse liver alcohol dehydrogenase.[7] When the horse liver alcohol dehydrogenase was preincubated with mepyrapone at 0°, the enzyme activity (subsequently analysed fluorimetrically at 24°) was reduced in proportion to the duration of the preincubation period (Fig. 3). However, ox liver glutamic dehydrogenase (NAD-linked) was inhibited not more than 10 per cent by 1 mM mepyrapone (G. H. Dodd, private communication).

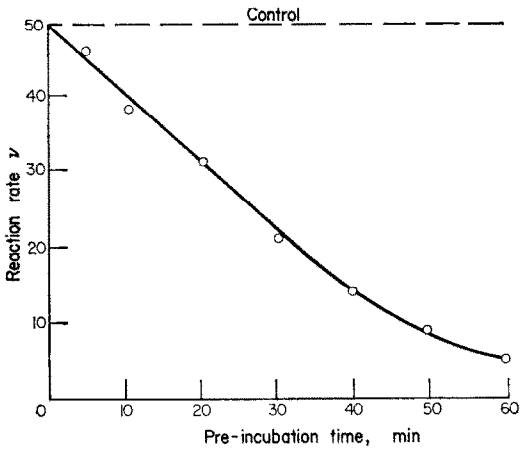

FIG. 3. The effects of pre-incubating liver alcohol dehydrogenase (horse) with SU 4885 (0·87 mM at 0°.

The rate v was measured at 8·1 mM ethanol, 0·5 mM NAD, 0·1 I phosphate (pH = 7·0) and $[E] = 0{\cdot}9\ \mu N$.[7]

Amphenone B, SU 8000 and SU 9055 which also inhibit steroid hydroxylation in the adrenal cortex[1] also inhibited cholesterol oxidation but not octanoate oxidation by mouse liver mitochondria. SU 8000 was the most potent of these three compounds (80 per cent inhibition at 5×10^{-5} M).

Studies with some hypocholesterolaemic drugs (SKF series)

The three drugs examined in detail, SKF 525A, SKF 2314 and SKF 3301, inhibited sterol oxidation but stimulated (straight-chain) fatty acid oxidation. In contrast to mepyrapone, these drugs (especially SKF 525A) inhibited the oxidation of one branched-chain sterol acid, cholestenoic acid, but did not affect the oxidation of 2-methyloctanoate.

The acid, SKF 2314 and the ether, SKF 3301, were consistently more potent inhibitors of 26-hydroxycholesterol oxidation by intact mitochondria than of cholestenoate oxidation. The ester SKF 525A was approximately equipotent as an inhibitor of the oxidation of both 26-hydroxycholesterol and cholestenoate.

SKF 525A stimulated palmitate oxidation (cf. octanoate oxidation) by mouse liver and beef heart mitochondria. In this respect it mimicked DL-carnitine over the same concentration range (0·25–2·0 mM). Figure 4 shows the effects of SKF 525A

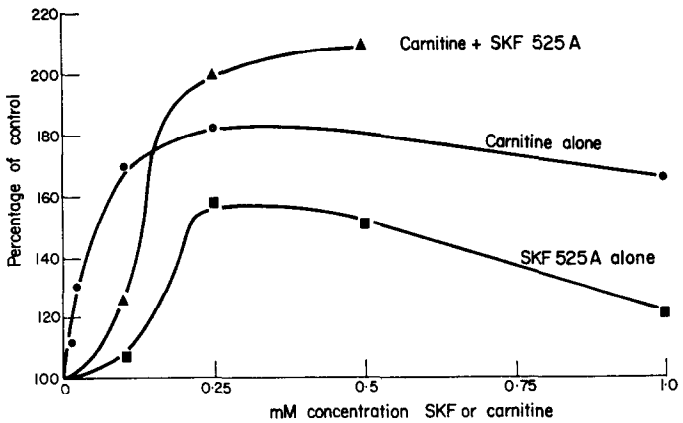

FIG. 4. The effects of adding SKF 525A and carnitine separately and together on [1-^{14}C]-palmitate oxidation to $^{14}CO_2$ by beef-heart mitochondria.

Results are expressed as percentages of the control (no drug added) and are averages of three separate experiments Mitochondrial protein = 25 mg/incubation. Incubation conditions are described in the text and by Dean and Whitehouse.[7]

and DL-carnitine, added singly or together, on palmitate oxidation by beef heart mitochondria. The meagre stimulation on adding these two compounds together (at carnitine concentrations less than 0·25 mM) suggests that SKF 525A might possibly antagonize carnitine, but this antagonism was overcome at higher carnitine concentrations.

SKF 525A and SKF 2314 did not inhibit horse liver alcohol dehydrogenase or 26-sterol dehydrogenase.

DISCUSSION

It would appear from these studies that these particular drugs are able to inhibit both hydroxylation and oxidation reactions in liver mitochondria involving sterol substrates. Furthermore mepyrapone may inhibit other NAD-linked dehydrogenases not involving steroids.

The present data would indicate that 26- and 24-hydroxylases (implicated in the metabolism of 7α-hydroxycholesterol and cholestenoate) are relatively insensitive to mepyrapone and that the prime effect of this drug on cholesterol oxidation is on the 26-hydroxysterol dehydrogenase.

Our findings again emphasize that SKF 525A is a most remarkable drug as regards the number of enzyme systems that it can inhibit (or stimulate).

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REFERENCES

1. R. GRANT, J. J. CHART and A. A. RENZI, *A. Rev. Pharmac.* **3**, 121 (1963).
2. W. HOLMES, in *Lipid Pharmacology* (Ed. R. PAOLETTI), p. 132. Academic Press, New York (1964).
3. H. DANIELSSON, *J. lipid Res.* **1**, 335 (1963).
4. P. D. G. DEAN and M. W. WHITEHOUSE, *Biochem. J.* **98**, 410 (1966).
5. P. D. G. DEAN and M. W. WHITEHOUSE, *Biochem. J.* accepted for publication (1967).
6. D. H. WILLIAMSON, J. H. MELLANBY and H. A. KREBS, *Biochem. J.* **80**, 82 (1962).
7. K. DALZIEL, *Biochem. J.* **84**, 244 (1962).