The Antilipolytic Effects of 3,5-Dimethylisoxazole and Possible Carboxylic Acid Metabolites

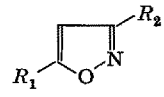
It has previously been reported that 3,5-dimethylisoxazole (3,5-DMI) reduced the fasting levels of plasma-free fatty acids (FFA) in the rat[1]. That this reduction in plasma FFA was due to reduced FFA release from adipose tissue was evidenced by the observation that the specific activity of plasma FFA after labeled palmitate administration was increased by 3,5-DMI.

Considerable evidence has been amassed to support the hypothesis that the hypoglycemic effects of a related heterocyclic compound, 3,5-dimethylpyrazole, are due to an active metabolite, 3-methylpyrazole-5-carboxylic acid[2], although the major metabolite is conjugated 4-hydroxy-3,5-dimethylpyrazole[3]. The corresponding isoxazole carboxylic acid, 3-methylisoxazole-5-carboxylic acid, has been suggested as an active metabolite that accounts for the antilipolytic and hypoglycemic activity of 3,5-DMI[4]. This report describes the effects of 3,5-DMI and its possible carboxylic acid metabolites on adipose tissue lipolysis in vitro and plasma FFA levels in dogs. The results indicate that, of the compounds studied, only 3-methylisoxazole-5-carboxylic acid (3-MIC) is active enough to explain the pronounced effects of 3,5-DMI on fat metabolism in intact rats and dogs.

The inhibition of norepinephrine-induced lipolysis was studied with epididymal adipose tissue taken from Sprague-Dawley rats, 160–200 g, fed ad libitum. The tissue was placed freshly aerated (95% O_2 to 5% CO_2) Krebs–Ringer bicarbonate buffer, pH 7.4, and minced into pieces weighing approximately 10 mg each. Each experimental flask contained 3 ml of aerated Krebs–Ringer bicarbonate buffer and 200 ± 3 mg of adipose tissue. Bovine plasma albumin at a final concentration of 1% was added to the buffer system. Sufficient norepinephrine (20–30 ng/ml) was added to the incubation mixture to cause a 50% of maximum release of FFA into the medium. The flasks were stoppered, aerated and shaken for 3 h on a Dubnoff metabolic shaker at 37 °C. Aliquots of the incubation medium were removed for FFA analysis by the DOLE procedure[5] and the effects of inhibitors expressed as % inhibition of the FFA release caused by norepinephrine.

Adult, mongrel dogs (8–14 kg) of both sexes were given i.v. doses (10 mg/kg) of the experimental compounds and changes in plasma FFA were monitored over an 8-h period. All solutions were administered at pH 7.0. Blood samples were withdrawn from the jugular vein and plasma FFA concentrations were determined by the methods of DOLE[5].

The following compounds were studied:



	R_1	R_2
3,5-DMI[a]	CH_3	CH_3
3-MIC[b]	COOH	CH_3
5-MIC[c]	CH_3	COOH
IDC[d]	COOH	COOH

[a] Purchased from K and K Laboratories, Inc., Plainview, New York, USA. [b] R. I. MELTZER, A. D. LEWIS, F. H. MCMILLAN, J. D. GRENZER, F. LEONARD and J. A. KING, J. Am. pharm. Ass., Sci. Edition *42*, 594 (1953). [c] J. B. WRIGHT, Belg. Pat. 627,420; Chem. Abstr. *60*, 12018 (1964). [d] C. MUSANTE, Gazz. chim. ital. *72*, 134 (1942).

The effects of 3,5-DMI and a series of potential metabolites on the norepinephrine-induced lipolysis in isolated adipose tissue were examined (Table). The parent compound, 3,5-DMI, was inactive as an inhibitor of lipolysis in this system. A possible metabolite, 3-MIC, was extremely effective, causing a significant inhibition at concentrations as low as $10^{-6}M$. 5-Methylisoxazole-3-carboxylic acid (5-MIC) was less active than 3-MIC by a factor of approximately 1000. Isoxazole-3,5-dicarboxylic acid (IDC), a compound in which both methyl groups of 3,5-DMI are oxidized to carboxyl groups, was without effect.

The same series of compounds was administered to adult mongrel dogs and their effects on fasting plasma FFA were monitored (Figure). Both 3,5-DMI and 3-MIC were extremely effective in depressing plasma FFA levels. A 75–80% decrease in plasma FFA values was observed and after 8 h these values were still depressed by 50%.

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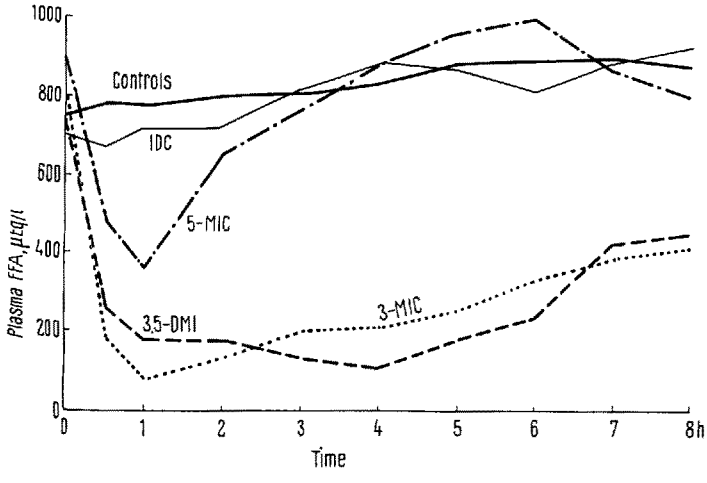

Time course of the effects of i.v. doses of 10 mg/kg of 3,5-dimethylisoxazole (3,5-DMI), 3-methylisoxazole-5-carboxylic acid (3-MIC), 5-methylisoxazole-3-carboxylic acid (5-MIC), and isoxazole-3,5-dicarboxylic acid (IDC) on the plasma FFA of fasting dogs. Each curve represents the mean response of 4 dogs.

The effects of 3,5-DMI and 3-MIC appear to be indistinguishable from each other. On the other hand, the effects of 5-MIC of plasma FFA were much less intense (50% reduction) and much less prolonged (normal in 2–3 h). As in the isolated adipose tissue system, IDC was ineffective in lowering plasma FFA values.

The lack of any effect of dimethylisoxazole on FFA release in an isolated adipose tissue system is demonstrated in this report and confirms the findings of others[4]. This observation together with the demonstrated effectiveness of 3,5-DMI in reducing plasma FFA levels in intact rats[6] and dogs (Figure) suggests that metabolism to an active agent is required. However, the unexpected finding that 3,5-DMI depresses plasma FFA in the eviscerated rat[6] indicates that the liver is probably not the site of the necessary metabolic step.

The findings reported here demonstrate that only 1 of the 3 possible carboxylic acid metabolites, 3-MIC, is potent enough in the isolated adipose tissue system and the intact dog to explain the effects of 3,5-DMI in vivo. The close similarity in time course of plasma FFA effects of both 3-MIC and 3,5-DMI provides additional evidence that 3-MIC is the active species and, further, suggests that the conversion of 3,5-DMI to 3-MIC is relatively efficient.

The lack of effectiveness of 5-MIC and IDC in both the adipose tissue system and the intact dog rules out these compounds as potential active metabolites of 3,5-DMI.

Percent inhibition of the norepinephrine-induced FFA release from isolated adipose tissue by 3,5-dimethylisoxazole, 3-methyl isoxazole-5-carboxylic acid, 5-methyl isoxazole-3-carboxylic acid and isoxazole-3,5-dicarboxylic acid

	Molar concentration				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
3,5-dimethyl-isoxazole	0	0	0	0	0
3-methylisoxazole-5-carboxylic acid	[a]81 ± 6	65 ± 6	62 ± 7	29 ± 3	0
5-methylisoxazole-3-carboxylic acid	23 ± 5	8 ± 3	0	0	0
isoxazole-3,5-di-carboxylic acid	0	0	0	0	0

[a] Mean $\pm$ S.D.; $n = 6$ experimental flasks.

Zusammenfassung. Von allen möglichen Isoxazolcarbonsäurenstoffwechselprodukten von 3,5-Dimethylisoxazol (3,5-DMI), die untersucht wurden, war nur die 3-Methylisoxazol-5-carbonsäure wirksam genug, um die auffallende Wirkung von 3,5-DMI auf unveresterte Fettsäurespiegel in normalen Hunden zu erklären.

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Fluorescence of Indole Derivatives

The identification and estimation of indolic compounds in biological material is of considerable interest. Due to the extremely low quantities in which several of these compounds occur in various tissues, fluorescence reactions are generally used. In this paper are described the different fluorescence properties of 5-methoxyindoles compared with other indolic compounds, using suitable solvent systems.

Early investigations of BOWMAN et al.[1] described a native fluorescence maximum at 330–360 nm in aqueous solution for many indoles after excitation at 275–296 nm. Using 3*N* HCl as a solvent, the 5-hydroxyindoles[2] and the 5-methoxyindoles[3] showed an additional fluorescence maximum at 550 nm. A systematic study of indole fluorescence at various pH values was made by WILLIAMS[4]. For a number of naturally occurring indoles a greater sensitivity could be reached by a chemical reaction resulting in a fluorescent product. The condensation of formaldehyde with indolealkylamines is used in histochemistry for the fluorescence microscopic localization of these compounds[5]. Serotonin[6,7] as well as its N,N-dimethyl derivative, bufotenine[8], can be determined with great sensitivity after reaction with ninhydrin. Recently, MAICKEL and MILLER[9] described the fluorescent properties of a number of indole derivatives after reaction with *o*-phthalaldehyde. Up to now no reliable technique existed for analytical differentiation between serotonin and melatonin.

According to LERNER[10] and MCISAAC[11] mammalian pineal glands appear to be a rich source of various indoles. During investigations on the presence of melatonin in pineal organs of lower vertebrates[12], the necessity was

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