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THE EFFECT OF LOCAL ANESTHETICS ON ARTERIAL LIPID METABOLISM

Inhibition of Sterol Esterification in Vitro

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Summary

The local anesthetics lidocaine, tetracaine, benzocaine and dibucaine were found to inhibit sterol esterification by acylCoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) in the microsomal fraction isolated from rabbit aortas. In arterial microsomes, the incorporation of [^{14}C]oleoylCoA into [^{14}C]steryl esters was inhibited in a dose-dependent way by the anesthetics over the concentration range 0.25—5.0 mM. The potency of inhibition was dibucaine > benzocaine > tetracaine > lidocaine > procaine with inhibition of about 85% occurring with 0.25 mM dibucaine. Sterol esterification to [^{14}C]oleic acid was also inhibited by the anesthetics in intact aortic tissue from the rabbit, dog, and rat. A detailed study of the effects of 5 mM lidocaine on lipid biosynthesis in the rabbit aorta in vitro revealed that lidocaine not only inhibited sterol esterification to [^{14}C]oleate but stimulated [^{14}C]oleate incorporation into glycerides.

Key words: *AcylCoA:cholesterol acyltransferase (EC 2.3.1.26) — Aorta — Atherosclerosis — Local anesthetics — Phospholipid synthesis — Sterol esterification*

Introduction

Interest in studying arterial lipid metabolism arises in part from our need to understand more fully the contribution of in situ lipogenesis to the accumulation of arterial lipid during atherogenesis. Regulation of arterial lipid-synthesizing enzymes, particularly those whose activity is greatly enhanced, may be

beneficial in modifying the development of atherosclerosis. The studies presented here indicate that lidocaine and other local anesthetics are capable of inhibiting sterol esterification in arterial tissue segments and in isolated arterial microsomes. We have also observed that some local anesthetics inhibit arterial phospholipid synthesis as well. Since sterol esterification and phospholipid synthesis are enhanced in atheromatous tissue [1—4], these anesthetic agents may serve as useful tools to investigate the regulation of lipid synthesis in arterial tissue or cultures of cells derived from arteries.

Materials and Methods

Animals and tissues

Male New Zealand rabbits, initially weighing 2.5—3 kg, were housed as previously described [5] and fed a hypercholesterolemic diet consisting of a pelleted chow (Purina Rabbit Chow) containing 3% peanut oil and 1% cholesterol by weight [5]. The rats (250—275 g) were normal males (Upjohn : TUC(SD)spf) which were fed a stock colony diet. The dogs used were 3—5-yr-old mongrel males obtained locally through a licensed supplier. The animals were killed by exsanguination under light pentobarbital anesthesia and their aortas quickly excised. The aortas were rinsed in chilled 0.9% NaCl solution and stripped free of adventitial tissue.

Incubations

Segments from rabbit aortas were incubated for 3 h at 37°C in 17 ml of a medium consisting of Medium 199 (Grand Island Biological Co., NY) and normal rabbit serum in the ratio of 1:1 (v/v) [6]. The medium contained streptomycin (50 μg/ml) and penicillin (50 units/ml) plus 0.23 μCi/ml of [1-^{14}C]oleic acid (sp. act. 56 mCi/mmol) which was obtained from New England Nuclear Corp., MA. Segments of dog aortas were incubated in 6 ml of Krebs—Ringer—bicarbonate buffer (pH 7.4) for 3 h at 37°C. The medium contained penicillin and streptomycin as above, plus [1-^{14}C]oleic acid (0.5 μCi/ml). Rat aortas were incubated in 3 ml of Krebs—Ringer—bicarbonate buffer (pH 7.4) containing 1 μCi/ml of [1-^{14}C]oleic acid. Local anesthetics (lidocaine—HCL from Sterling Organics, NY, and dibucaine—HCL, procaine—HCL, tetracaine—HCL, and benzocaine from Sigma Chemical Co., MO) were added to the incubations dissolved in a portion of the incubation medium except for benzocaine which was added dissolved in 25—50 μl of diethyl ether.

Assay of microsomal acylCoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26)

The microsomal fraction from arteries was isolated from arterial homogenates prepared in 0.1 M phosphate buffer (pH 7.4) as previously described [7]. AcylCoA:cholesterol acyltransferase was assayed by following the incorporation of [^{14}C]oleoylCoA into [^{14}C]steryl esters. The assay system consisted of a total volume of 345 μl of 0.1 M phosphate buffer (pH 7.4) containing 0.20—0.25 mg microsomal protein and 2.6×10^5 dpm of [1-^{14}C]oleoylCoA (sp. act. 49 mCi/mmol, New England Nuclear Corp., MA). Local anesthetics were introduced into the assay tubes (13 × 100 mm) dissolved in 15 μl saline except for benzocaine and procaine which were introduced in 25 μl of diethyl ether, and

acetone, respectively. Assays were run for 5 min at 37°C and then terminated by the addition of 2 ml of methanol. Blanks without enzyme and with boiled enzyme were included. Neither acetone nor diethyl ether affected the assays when used at levels up to 25 μl.

Analyses

Following incubation, arterial segments were rinsed in five 100-ml changes of 0.9% NaCl solution and then extracted by homogenization in $CHCL_3$-MeOH (2:1, v/v) [6]. The lipid extracts were fractionated by thin-layer chromatography (TLC) on silica gel G-coated plates in *n*-hexane:diethyl ether:acetic acid (146:50:4, v/v/v) and the various lipid fractions assayed for radioactivity; these procedures have been reported previously [6,8].

The samples used to assay microsomal acylCoA:cholesterol acyltransferase were evaporated to ca. 0.1 ml under N_2 at 37°C and extracted with $CHCL_3$-MeOH as above. The lipid extracts were fractionated by TLC as above and the steryl ester fraction was taken for radioactive assay.

Protein was measured in the microsomal preparations by the method of Lowry et al. [9].

Results

Table 1 shows the effect of lidocaine in vitro on the incorporation of [1-^{14}C]oleic acid into lipids of arterial tissue from rabbits fed the atherogenic diet for 8 wk. At a final concentration of 5 mM in the incubation medium, lidocaine significantly increased incorporation of [^{14}C]oleate into the diglyceride and triglyceride fractions ($P < 0.01$ and $P < 0.05$, respectively) but significantly reduced incorporation into steryl esters ($P < 0.001$). Overall, there was a net decrease ($P < 0.05$) in the incorporation of [^{14}C]oleate into total lipids in the presence of lidocaine (Table 1). This net decrease was not a reflection of reduced [^{14}C]oleate uptake since tissue levels of [^{14}C]free fatty acid were

TABLE 1

EFFECT OF LIDOCAINE ON THE INCORPORATION OF [^{14}C]OLEATE INTO LIPIDS OF AORTAS FROM CHOLESTEROL-FED RABBITS IN VITRO (dpm/g wet wt).

Aortas from 11 rabbits fed the atherogenic diet (1% cholesterol plus 3% peanut oil) for 8 weeks were bissected longitudinally. One half was incubated in the presence of 5 mM lidocaine, the other half was incubated in the absence of lidocaine (Control). Incubations were for 3 h at 37°C in 17 ml of a medium composed of normal rabbit serum and medium M-199 (1 : 1, v/v), which contained 0.23 μCi/ml of [1-^{14}C]oleate.

Arterial lipids were extracted with chloroform—methanol (2 : 1, v/v) and fractionated by thin layer chromatography as described in Methods.

	[^{14}C]Phospholipid	[^{14}C]Diglyceride	[^{14}C]Triglyceride	[^{14}C]Steryl ester	[^{14}C]Total
Control	287 250 ± 60 350 [a]	11 500 ± 1625	50 600 ± 10 950	60 225 ± 11 400	409 575 ± 57 875
Lidocaine (5 mM)	235 700 ± 45 900	18 775 ± 1575	73 547 ± 8525	4125 ± 1325	332 168 ± 49 550
	NS [b]	$P < 0.01$	$P < 0.05$	$P < 0.001$	$P < 0.05$

[a] Values are means ± SEM of data derived from the 11 aortas.

[b] Data from each pair of tissue segments were analyzed using Student's paired *t*-test.

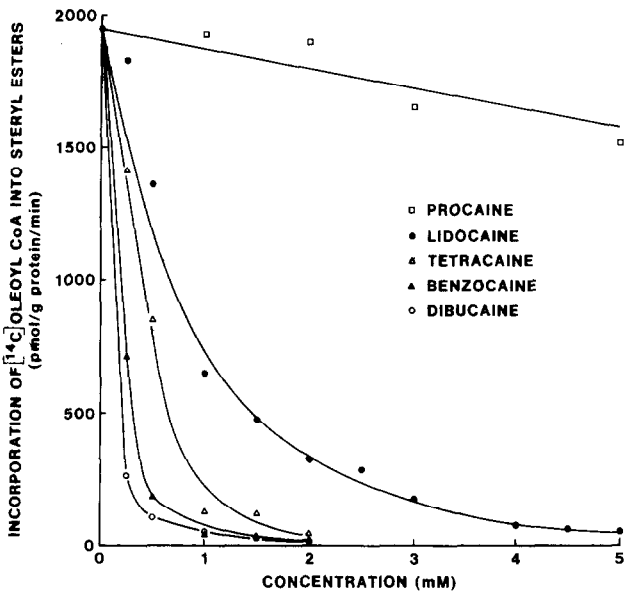

Fig. 1. The effect of various local anesthetics on the incorporation of [1-^{14}C]oleoylCoA into sterol esters in isolated arterial microsomes from the aortas of cholesterol-fed rabbits. Isolation of arterial microsomes and assay of acylCoA:cholesterol acyltransferase with [^{14}C]oleoylCoA is described in detail under Methods.

higher in the tissue segments incubated with lidocaine than in the control segments (136 000 ± 23 000 vs 109 000 ± 25 000 dpm/g wet wt, $P < 0.01$, n = 11). Of particular interest in the present studies was the observed inhibition of steryl ester synthesis in the presence of lidocaine. This observation was investigated in more detail by assaying acylCoA:cholesterol acyltransferase in the microsomal fraction isolated from rabbit aortas (Fig. 1). As shown in Fig. 1, the enzyme was assayed in the presence of lidocaine over the concentration range 0.25—5.0 mM. Lidocaine was inhibitory at all concentrations with 50% inhibition occurring between 0.5 and 1 mM; 90% inhibition was achieved at lidocaine concentrations of about 3 mM. Other local anesthetics were studied and found to be inhibitory as well (Fig. 1). Procaine gave a weak inhibition of the enzyme which reached about 20% at 5 mM; levels below 2 mM did not affect the enzyme. In contrast to procaine, tetracaine, benzocaine, and dibucaine were stronger inhibitors of acylCoA:cholesterol acyltransferase than lidocaine and each resulted in almost complete inhibition of the enzyme at a level of 2 mM. The order of inhibitory potency of the anesthetics on microsomal acylCoA: cholesterol acyltransferase as observed in Fig. 1 (dibucaine > benzocaine > tetracaine > lidocaine > procaine) was a consistent observation in all such studies conducted.

The inhibitory effect of local anesthetics on acylCoA:cholesterol acyltransferase in rabbit aorta (Table 1) is not species-specific since the effect could be demonstrated in dog and rat arteries as well (Table 2). Esterification of [^{14}C]-oleate to cholesterol in segments of dog aortic intima—media incubated in vitro with 5 mM lidocaine, tetracaine, and dibucaine was inhibited 72%, 87%, and 89%, respectively (Table 2); the relative order in which the various agents inhib-

TABLE 2

EFFECTS OF LOCAL ANESTHETICS ON THE INCORPORATION OF [^{14}C]OLEATE INTO STERYL ESTERS OF THE NORMAL DOG AND RAT AORTAS

The lower thoracic region of aortas from 2 male mongrel dogs was stripped free of adventitia and the resulting intima—media cut into segments of 400 mg each for incubation. The tissues were incubated for 3 h at 37°C in 6 ml of Krebs—Ringer—bicarbonate buffer (pH 7.4) containing 3 μCi of [1-^{14}C]oleic acid (sp. act. 56 mCi/mmol). Aortas from male rats weighing 240—270 g were removed, stripped of adventitial debris and incubated as pools of 2 aortas in 3 ml of Krebs—Ringer—bicarbonate buffer with 3 μCi [1-^{14}C]oleate as above.

Anesthetics were present in the incubations at a final concentration of 3 mM or 5 mM as indicated. The tissues were extracted with $CHCl_3$-MeOH (2 : 1, v/v) and the [^{14}C]steryl esters isolated and measured as described under Methods.

Source of arteries	Additions	dpm/g wet wt	% Control
Dog	None (control)	3570 [a]	100
	Lidocaine, 5 mM	1000	28
	Tetracaine, 5 mM	465	13
	Dibucaine, 5 mM	400	11
Rat	None (control)	2320 ± 565 [b]	100
	Lidocaine, 3 mM	800 ± 185	35 ± 8

[a,b] Values are the mean of 2 experiments using dog aortas or the means ± SEM of 3 experiments using rat aortas.

ited esterification was essentially the same as observed in Fig. 1 in isolated microsomes. The 72% inhibition of sterol esterification by 5 mM lidocaine in the dog aorta is somewhat less than that observed at this concentration in the rabbit aortas (90%, Table 1) and may reflect differences in penetration of the anesthetics as a result of differences in tissue thickness between dog vs rabbit aorta. In similar studies using rat aortas, lidocaine inhibited the incorporation of [^{14}C]oleate into [^{14}C]steryl esters. At a level of 3 mM, the only level used with rat aortas, inhibition of esterification was about 65% (Table 2). The possible effect of the local anesthetics on other aspects of lipid synthesis in the dog and rat aorta was not investigated in these studies.

Discussion

Local anesthetics are known to influence, in cell-free systems, heterogeneous catalysis by a number of lipid-metabolizing enzymes such as Ca^{2+}-dependent phospholipases [10—13], triacylglycerol lipases [14], and cholesterol esterase [15]. Various local anesthetics have also been shown to stimulate phosphatidyl inositol synthesis in pineal gland [16] and vagus nerve preparations [17], and to inhibit hormone-stimulated lipolysis in isolated fat cells [18] and to inhibit sterol synthesis in rat C_6 glioma cells [19]. The present studies were undertaken to investigate the possible effects of local anesthetics on lipid metabolism in arterial tissue. Studies on the enzymes of arterial lipid metabolism are of interest because of the role they may perform in the development or reversal of the atherogenic process. Early metabolic changes occurring during the development of atherosclerosis include enhanced phospholipid synthesis [3,4] and enhanced synthesis of steryl esters [1,2] which contribute to the accumulation of these

lipids in the artery. The present studies which show for the first time that local anesthetics are capable of inhibiting synthesis of arterial steryl esters are of particular interest since the anesthetics may be useful as tools for probing the manner in which sterol esterification is regulated in vivo. The data also emphasize the fact that arterial lipid-metabolizing enzymes lend themselves to selective inhibition; the inhibition of steryl ester synthesis by lidocaine is not the result of a general inhibition of enzymes since there is a simultaneous increase in the synthesis of glycerides but little or no effect on phospholipid synthesis. The mechanism by which the local anesthetics affect the various enzymes reflected in these studies is an open question. While a direct effect of the local anesthetics on various enzymes [12] or an interaction of the anesthetics with substrates [20] is possible, modification of arterial enzyme activities observed here could arise indirectly as a consequence of membrane-induced effects, particularly since glycerides, and steryl esters require the participation of membrane-bound (microsomal) enzymes for their synthesis [7,21—23]. The ability of local anesthetics to alter membrane—lipid fluidity is well documented [24—26] and may be important to the observations here since alterations in membrane—lipid fluidity are known to modify the activity of various membrane-bound enzymes in other systems [27—31]. Since the response of membrane-bound enzymes to changes in membrane fluidity cannot be known *a priori* and may be manifest by either increases or decreases in enzyme activity, the differing responses of glyceride synthesis vs steryl ester synthesis observed here are not inconsistent with proposed membrane effects. In fact, the relative inhibitory potency of the local anesthetics on sterol esterification in arterial preparations (Fig. 1 and Table 2) is similar to their relative potency in blocking nerve conduction [24,32]. Increased glyceride synthesis in the presence of lidocaine could also be explained by an increased availability of fatty acid since a decrease in sterol esterification would tend to spare fatty acids for other pathways. This explanation seems less likely to account for the stimulated glyceride synthesis since, as pointed out earlier in the text, control segments of artery accumulated in excess of 100,000 dpm [^{14}C]free fatty acid/g wet wt.

During the course of these studies, we did not undertake to measure cholesterol ester hydrolase (EC 3.1.1.13) which is known to be inhibited by local anesthetics in liver [15]. Inhibition of the enzyme in the present studies would not be expected to significantly alter the net yield of esterified cholesterol in the ACAT assays. Under experimental conditions similar to those reported in Fig. 1, Brecher et al. [33] reported less than 5% hydrolysis of cholesterol oleate by isolated arterial microsomes in incubations of 60 min which is considerably longer than the 5-min assays used here to measure ACAT.

Other local anesthetics are presently being evaluated for their effects on arterial lipid synthesis.

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