Table 1. Effects of choline analogs and cholinergic drugs on [^{3}H]-QNB binding in rat brain*

Compound	IC_{50} (μM)	
Anticholinergic		
Scopolamine	0.014 ± 0.008	(4)
Cholinergic		
Oxotremorine	0.540, 0.600	(2)
Arecoline	8.60 ± 2.5	(4)
Pilocarpine	4.8 ± 1.7	(3)
Carbachol	89.7 ± 3.2	(3)
Choline analogs		
Choline	1,800 ± 600	(4)
Betaine aldehyde	2,800 ± 800	(3)
Glycerylphosphorylcholine	6,200 − > 10,000	(4)†
Dimethylaminoethanol‡	7,900 ± 3,300	(3)
Cytidinediphosphocholine	>10,000	
Phosphorylcholine	>10,000	

* Results are given as the means ± S.D. with the number of observations in parentheses. Each compound was tested at two to four concentrations in triplicate for each separate IC_{50} determination. [^{3}H]-QNB (sp. act. 29.4 Ci/mmole, New England Nuclear, Boston, MA.) was present at a final concentration of 60 pM. Nonspecific binding was determined using 100 μM oxotremorine.

† Glycerylphosphorylcholine was insoluble at the concentrations used and gave varying IC_{50} values.

‡ The acetamidobenzoic acid salt (Deaner) was used.

Because of the efficiency of the cellular uptake processes for choline in brain, the majority of this choline is likely to be intracellular, and therefore the concentration of choline at the cholinergic receptor would be expected to be far lower than the level determined in whole tissue.

Furthermore, it seems unlikely that a metabolite of choline, formed after the compound is administered, could be responsible for a direct cholinomimetic action, as none of the metabolites tested was as potent as choline in displacing QNB. In addition, the amounts of these metabolites found in the brain when [^{3}H]-choline is administered to animals is quite small [10].

The central cholinergic action of choline would appear, therefore, to be elicited by a mechanism other than that of direct stimulation of muscarinic cholinergic receptors. One such possibility in this regard is that choline might stimulate nicotinic cholinergic receptors, but this seems unlikely since its central effects can be blocked by atropine [11, 12]. Another possibility is that choline acts pre-synaptically to stimulate the synthesis and, presumably, the release of ACh from cholinergic neurons. This latter possibility is consistent with the observations that (1) choline administration causes an increase in the concentration of brain acetylcholine [9], (2) both the choline-induced increase in brain levels of ACh and the central cholinergic effects [11, 13] are blocked by inhibitors of acetylcholine synthesis, and (3) choline stimulates *in vitro* the rate of release of ACh in the heart [14].

Neuropsychopharmacology Section, Merck Institute for Therapeutic Research, West Point, PA 19486, *U.S.A.*

DEAN R. HAUBRICH
EDWIN A. RISLEY
MICHAEL WILLIAMS

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Clofibrate-induced increase in carnitine and acylcarnitine content in rats

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It has been shown that clofibrate administration markedly increases fatty acid oxidation in rat liver [1–5]. A corresponding increase of several enzyme activities connected to the fatty acid oxidation sequence is found [6, 7]. Previously, it has been briefly reported that carnitine content in rat liver is increased after clofibrate administration [3, 8]. Carnitine plays a major role in the transport of activated fatty acyl groups from cytosol to sites of β oxidation in the mitochondria. In general, the changes in liver carnitine appear to correlate with the rate of fatty acid oxidation [9, 10]. As a consequence, a regulatory role for carnitine in fatty acid oxidation has been postulated [9, 11]. In this study the effect of clofibrate on carnitine and its derivatives in different tissues and total body of the rat was investigated. This report provides also a clarification regarding the role of hepatic carnitine in the hypotriglyceridaemic effect of the drug.

Male Wistar rats weighing 200–300 g were used in the experiments. The animals were allowed free access to standard laboratory chow and water. The treatment consisted of daily subcutaneous injections of clofibrate (200 mg/kg body wt) for 14 days. Control rats received saline. Rats were killed by decapitation and blood collected in heparin. The heart, a piece of liver and of skeletal muscle (hind leg)

Table 1. Effect of clofibrate administration on carnitine and acylcarnitine content in rat plasma and tissues

		Carnitine	Acetyl carnitine	C_{3-9} acylcarnitines* (nmoles/ml or g wet wt)	Total acid-soluble carnitine	Acid-insoluble carnitine
Liver	Control	153 ± 15	87 ± 12	21 ± 26	261 ± 29	19 ± 3.7
	Clofibrate	845 ± 101‖	380 ± 41‖	334 ± 109‡	1560 ± 120‖	32 ± 4.2†
Heart	Control	608 ± 54	427 ± 38	324 ± 155	1360 ± 160	127 ± 19
	Clofibrate	800 ± 73†	478 ± 33	235 ± 83	1510 ± 70	113 ± 12
Skeletal muscle	Control	741 ± 77	92 ± 11	84 ± 95	917 ± 100	52 ± 6.3
	Clofibrate	743 ± 75	86 ± 15	162 ± 87	991 ± 43	54 ± 5.9
Plasma	Control	29 ± 1.5	14 ± 0.8	25 ± 3.2	68 ± 3.8	—
	Clofibrate	34 ± 2.4	19 ± 1.5‡	35 ± 3.6	87 ± 4.1§	—

* C_{3-9} acylcarnitines were calculated by subtracting free carnitine and acetylcarnitine from total acid-soluble carnitine.
Clofibrate (200 mg/kg body wt) was given once a day for 14 successive days. The results are the means ± S.E.M. for groups of six rats. Student's *t*-test was used to calculate significance.
† $P < 0.05$.
‡ $P < 0.02$.
§ $P < 0.01$.
‖ $P < 0.001$.

were quickly excised and freeze-clamped with aluminium blocks pre-cooled in liquid N_2. Perchloric acid extracts of plasma and tissue samples were prepared using the method described by Pearson and Tubbs [12]. The supernatant was analysed for carnitine and acetylcarnitine. Acid-soluble carnitine and acid-insoluble carnitine were determined as free carnitine after alkaline hydrolysis of the supernatant and the insoluble precipitate, respectively.

Carnitine was measured in a coupled enzymatic assay. The CoA-SH liberated in transacetylation between acetyl-CoA and carnitine was allowed to react further using 2-oxoglutarate dehydrogenase system as an indicator enzyme [13]. The final reaction volume was 1.0 ml and contained 35 mM arsenate buffer, pH 7.2, 0.5 mM 2-mercaptoethanol, 1 mM EDTA, 350 μM 2-oxoglutarate, 150 μM acetyl-CoA and 10 mU 2-oxoglutarate dehydrogenase*. The increase in $A_{340-400}$ was monitored with a double-beam spectrophotometer after addition of 1 unit of carnitine acetyltransferase. 2-Oxoglutarate dehydrogenase was isolated from pig heart as described by Sanadi *et al.* [14]. The concentrations of the (−)-carnitine standards were measured by the DTNB-method [13]. Acetylcarnitine was determined by a standard procedure [13]. Assays for protein and carnitine acetyltransferase (EC 2.3.1.7) and the isolation method for mitochondria were as described previously [15].

The concentrations of carnitine, acetylcarnitine, C_{3-9} acylcarnitines, total acid-soluble carnitine (i.e. free carnitine plus acylcarnitines C_2–C_9) and acid-insoluble acylcarnitines ($C_{>9}$) in different rat tissues and plasma are shown in Table 1. Clofibrate treatment induced an increase of many fold in the hepatic concentration of total carnitine. The increase in the hepatic concentration of total acid-soluble carnitine was partly due to a rise in free carnitine and acetylcarnitine. However, the drug increased the concentration of other short chain (C_3–C_9) acylcarnitines, as well. It has been shown that, in addition to acetylcarnitine, normal rat liver contains appreciable quantities of other short chain acylcarnitines [16]. Acid-insoluble acylcarnitines were elevated to a smaller extent. The increase in hepatic carnitine found in this study is more pronounced than in fasting and diabetes.

The treatment slightly increased the concentration of free carnitine in heart. The drug did not induce statistically significant changes in other carnitine compounds in heart. No changes were found in skeletal muscle. The levels of acetylcarnitine and total acid-soluble carnitine in plasma showed a significant increase after clofibrate treatment.

It is known that clofibrate increases the concentration of CoA and its fatty acyl esters in liver [17]. The change in CoA compounds occurs with a pattern similar to the changes in carnitine and its esters found in the present study. Thus, it is probable that the rise in acid-soluble acylcarnitines reflects increased hepatic content of CoA and short chain acyl-CoA. Acetylcarnitine can be released from the liver [18]. Therefore, it is presumed that in livers of clofibrate-treated rats part of the acetyl pressure generated by the accelerated fatty acid oxidation is relieved by the formation and release of acetylcarnitine.

The striking increase in the content of carnitine and its derivatives in liver is also reflected in total body carnitine (Table 2). Total carnitine was measured in perchloric acid extracts of carcasses prepared by removing the head, feet,

* One unit (U) is defined as 1 μmol of substrate converted/min at 25°.

Table 2. Effect of clofibrate administration on liver and whole body carnitine content

	Total acid-soluble carnitine		
	Liver (μmoles/100 g final body wt)	Total body (μmoles/100 g final body wt)	Total body (μmoles/100 g initial body wt)
Control	0.86 ± 0.09	40.0 ± 10.5	47.9 ± 10.7
Clofibrate	7.6 ± 0.63‡	47.5 ± 2.4†	53.2 ± 2.1*

Mean values ± S.E.M. are from six animals in both groups. Treatment period 14 days. Statistics: see Table 1.
* $P < 0.05$.
† $P < 0.02$.
‡ $P < 0.001$.

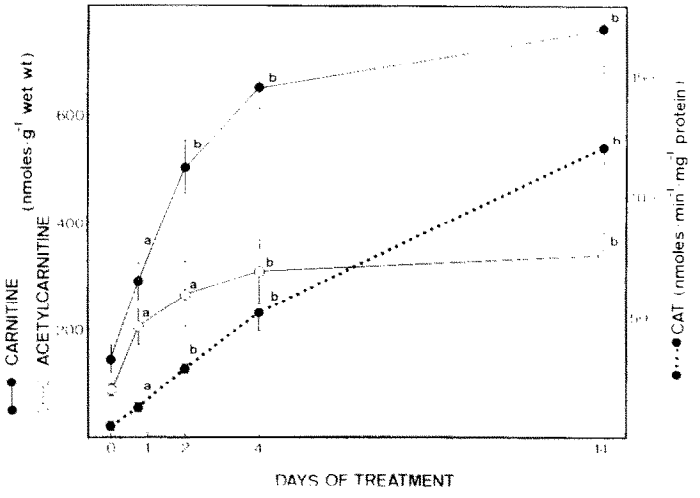

Fig. 1. The time course of the clofibrate-induced changes in hepatic carnitine and acetylcarnitine. Comparison with the mitochondrial activity of carnitine acetyltransferase (CAT). Rats were given clofibrate (200 mg/kg body wt) once a day.

Values are means ± S.E.M. of 3–6 rats at each time period.

[a]Statistically different from the control group, $P < 0.01$, [b]$P < 0.001$.

skin, tail and intestinal tract [19]. The small contribution of acid-insoluble acylcarnitines was excluded. It is worth noting that part of the rise in the hepatic content of carnitine is due to the clofibrate-induced increase in liver to body weight ratio (means 0.033 and 0.048 in the control and clofibrate groups, respectively). Because the rats in the clofibrate group gained weight slightly less during the treatment period than the controls, total body carnitine is also expressed per 100 g of initial body weight. The clofibrate-induced increase is statistically significant also when the calculation is based on the initial weights indicating that the rise is due to altered rate of synthesis or/and elimination of carnitine. Thus, it is evident that the rise in carnitine in liver is not merely due to a redistribution in the body.

To gain some insight into the nature of the changes in liver carnitine the time-response and dose-response relations were studied. The concentrations of free carnitine and acetylcarnitine were compared to the activity of mitochondrial carnitine acetyltransferase which is highly responsive to clofibrate administration [6, 8]. The temporal changes produced by the administration of clofibrate (200 mg/kg body wt) are presented in Fig. 1. The mean values of the control rats remained virtually constant during the treatment period (data not shown). In the clofibrate group statistically significant changes were found already 18 hr after the first injection. The enzyme activity increased in parallel with carnitine concentrations. Similar parallelism was found in the dose-response experiment (Fig. 2).

It must be noted that the smallest dose of clofibrate (30 mg/100 g body wt) used in the dose-response experiment had no effect on the hepatic concentration of carnitine and acetylcarnitine (Fig. 2). However, this dose effectively

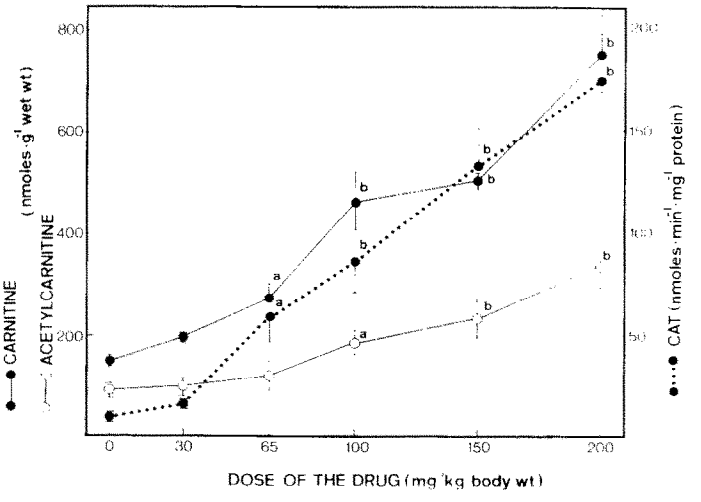

Fig. 2. Effect of different doses of clofibrate on hepatic carnitine and acetylcarnitine content. Comparison with the mitochondrial activity of carnitine acetyltransferase (CAT). Rats were injected daily with clofibrate for 14 days.

Values represent mean ± S.E.M. for groups of 3–8 rats.

[a]Statistically different from the control group, $P < 0.01$, [b]$P < 0.001$.

decreased the serum concentration of triglycerides both in rats made hypertriglyceridaemic with fructose [15] and in normal rats deprived of food 12 hr before sacrifice (own unpublished data). Thus it is probable that the clofibrate-induced increase in carnitine compounds is not connected to the hypotriglyceridaemic effect of the drug. This is further supported by a similar lack of correlation between hepatic activities of carnitine acyltransferases and serum triglyceride level in clofibrate-treated rats [15].

The results demonstrate increased concentrations of carnitine and short chain length acylcarnitines in livers and plasma of clofibrate-treated rats. The changes in liver were intimately associated with the activity of carnitine acetyltransferase. The increased hepatic content of carnitine cannot be explained as a redistribution in the body because the total body carnitine was also increased. The dose-response relations do not support the view that hepatic carnitine has a role in the hypotriglyceridaemic effect of clofibrate.

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Department of Medical Chemistry,
University of Helsinki,
Siltavuorenpenger 10A,
SF-00170 Helsinki 17,
Finland

MATTI T. KÄHÖNEN

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Effect of suloctidil* on Na^+/K^+ ATPase activity and on membrane fluidity in rat brain synaptosomes

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It has been clearly demonstrated that Na^+/K^+ ATPase is involved in the active transport of Na^+ and K^+ across plasma membranes [1]. At the level of the central nervous system, the Na^+/K^+ ATPase is concentrated in synaptic membranes [2]. Due to this localization, the enzyme is sensitive to modifications of the membrane structure produced by various exogenous or endogenous factors [3, 4]. As far as drugs are concerned, general and local anaesthetics and phenotiazines have been shown to inhibit Na^+/K^+ ATPase activity [5] and to fluidify the lipid matrix of the synaptosomes [6, 7]. However, with our present knowledge no clearcut relationships have yet been established between these two parameters [7, 8]. It has nevertheless been suggested that variations in the activity of the Na^+/K^+ ATPase would markedly alter information of transmission between nerve cells [9].

In this paper, the effects of suloctidil (and related compounds), chlorpromazine (CPZ) and ouabain on Na^+/K^+ ATPase activity and on membrane fluidity have been studied in rat brain synaptosomes. Suloctidil is an amino-alcohol endowed namely with vasoactive properties [10, 11] that could be related to a membrane effect.

Male Sprague–Dawley rats were used. Chemicals and their suppliers were as follows: suloctidil (erythro-*p*-(isopropyl-thio)-α[1-octylamino-ethyl] benzyl alcohol), CP 1136 S (erythro-*p*-(isopropylthio)-α-(1-aminoethyl) benzyl alcohol) and CP 894 S (erythro-*p*-(isopropylthio)-α-[1-(octylthio)-ethyl] benzyl alcohol) from Continental Pharma S.A., Brussels, Belgium, chlorpromazine from Rhone Poulenc S.A. and ouabain (g-Strophantin) from Boehringer, Mannheim GmbH (W. Germany).

Synaptosomes were prepared from rat brains using the procedure of Gray and Whittaker [12].

ATPase (Na^+ and K^+ activated, ouabain sensitive adenosine-5′-triphosphatase, EC 3.6.1.3) activity has been assayed by measurements of the free inorganic phosphate according to the colorimetric method of Fiske and Subbarow [13]. The synaptosome suspension was diluted 80 times in a medium containing 115 mM NaCl, 20 mM KCl, 2 mM MgCl, 1 mM EDTA and 40 mM Tris–maleate. The pH was adjusted to 7.4. 1.5 ml of the reaction mixture was incubated with 25 μl of DMSO either containing or not containing the drug to be investigated. The ATPase activity was initiated by the addition of 250 μl of a solution of ATP