Biochemical Pharmacology, Vol. 33, No. 17, pp. 2815–2817, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Effects of aspirin and salicylic acid on lecithin-cholesterol acyltransferase and cholesterol content in rat serum

(*Received* 6 *September* 1983; *accepted* 31 *January* 1984)

Human plasma and rat serum contain lecithin-cholesterol acyltransferase (EC 2.3.1.43) (LCAT), which catalyzes the formation of cholesterol ester and lysolecithin from cholesterol and lecithin, respectively, on high density lipoproteins ($1.063 < d < 1.210\ g/cm^3$, HDL) [1, 2]. Since lysolecithin produced by this reaction is a potent feedback inhibitor, the acyltransferase reaction requires HDL as a cofactor lipoprotein and albumin for the elimination of lysolecithin from HDL [3, 4]. On the other hand, aspirin and salicylic acid also bind strongly to serum albumin in circulation [5, 6]. In addition, aspirin is known to acetylate serum albumin under physiological conditions [7]. Therefore, it is of interest to investigate the effects of aspirin and salicylic acid as albumin binders on the acyltransferase reaction. Furthermore, the effects of aspirin and salicylic acid on cholesterol metabolism in circulation are little known. In this paper, we investigated the alteration of LCAT activity and cholesterol content in serum of rats administered aspirin and salicylic acid.

Male albino rats of the Wistar strain, weighing 190–230 g, were used. Some rats were starved for 48 hr. Also, some starved rats were fed a commercial diet (Oriental Yeast Co., Tokyo, Japan). Food was removed from the cages of all rats at 9:00 a.m. and returned in the evening (6:00 p.m.). To test the effects of aspirin and salicylic acid, rats were divided into three groups (three rats/each group) and given orally 0.5 ml of 1% sodium carboxymethylcellulose (CMC) solution, 0.5 ml of 1% CMC solution containing aspirin (50 mg/kg body), and 0.5 ml of 1% CMC solution containing salicylic acid (50 mg/kg body), respectively, three times (9:00 a.m., 3:00 p.m. and 9:00 p.m.) a day for 3 days. In addition, to test the effect of cycloheximide with aspirin or salicylic acid, cycloheximide (2 mg/kg body in saline) was injected intraperitoneally 1 hr prior to the administration of aspirin or salicylic acid. Aspirin (50 mg/kg body) or salicylic acid (50 mk/kg body) was administered orally three times (9:00 p.m., 3:00 a.m. and 9:00 a.m.) for 12 hr. At 2 hr after the final administration, blood was drawn from these rats by cardiac puncture into syringes and was centrifuged for 15 min at 3500 r.p.m. at 4° or less. Rat serum obtained was diluted with Tris–HCl buffer (pH 7.4, ionic strength 0.1) to give a protein content of 60 mg/ml. The protein content was determined by the procedure of Lowry *et al.* [8]. As the substrate for LCAT reaction, lecithin/[7 – ^{3}H(N)] cholesterol (New England Nuclear Corp., Boston MA, U.S.A.) liposome solution with a molar ratio of 1.0 was prepared by the method of Batzri and Korn [9]. The amounts of radioactivity and free cholesterol in 1 ml of liposome solution were 1 μCi/0.3 μmole. The incubation mixture for the enzyme assay contained 0.1 ml of substrate solution and 0.2 ml of rat serum (60 mg protein/ml). The final volume was adjusted to 0.5 ml with Tris–HCl buffer (pH 7.4). The incubation was carried out at 37° for 3 hr with mechanical shaking. After incubation, extraction and separation of lipids and measurement of radioactivity were determined as described previously [10]. The determination of free cholesterol in rat serum was performed using a kit (Free Cholesterol C-Test Wako, Wako Chemical Ind., Osaka, Japan) based on an enzymatic reaction. For the determination of total cholesterol in rat serum, total lipid extracted from rat serum by the procedure of Folch *et al.* [11] was hydrolyzed with 5% methanolic-KOH at 60° for 1 hr. Then, total cholesterol was extracted with *n*-hexane and was determined by the procedure of Muesing and Nishida [12].

In general, the usual single dose of aspirin or salicylic acid in adults is 0.5 to 1.5 g. This may be repeated every 4 hr. However, for maximal suppression of rheumatic inflammation, a total daily dosage of 5–8 g is given to maintain a plasma salicylate level of 25–35 mg/100 ml. In the present experiments, we used a considerably higher total daily dosage of these drugs. The acyltransferase activities in rat serum and human plasma for a lecithin/cholesterol liposome solution with a molar ratio of 1.0 increased linearly with time up to 3 hr under the present experimental conditions.

As shown in Table 1, the *in vivo* acyltransferase activity in serum of rats administered aspirin or salicylic acid was elevated 22–28%, compared with that in control rats. The elevation of acyltransferase activity may have been related to a direct action of aspirin or salicylic acid on the enzyme, an inductive action of aspirin or salicylic acid on the biosynthesis of proteins such as the enzyme and cofactor lipoprotein, and/or a decrease of free cholesterol content in rat serum. Experiments *in vitro* showed that when rat serum was incubated with concentrations of aspirin or salicylic acid ranging from 1×10^{-6} M to 1×10^{-3} M, acyltransferase activity was not stimulated by aspirin or salicylic acid and was depressed at concentrations above 1×10^{-4} M aspirin or salicylic acid (date not shown); the inhibitory action of salicylic acid on the acyltransferase reaction was slightly higher than that of aspirin. Whether the inhibitory action of aspirin on the acyltransferase reaction *in vitro* was due to the acylation of proteins such as the enzyme, lipoproteins and albumin is unclear. Since the acyltransferase reaction was inhibited by salicylic acid, as well as by aspirin *in vitro*, the inhibitory action of aspirin on the acyltransferase reaction may be little related to the acylation of the proteins.

Furthermore, we examined the acyltransferase activity in serum of rats administered cycloheximide with or without aspirin. As shown in Table 2, the acyltransferase activity in serum of rats administered cycloheximide was depressed to 44–56% of control rats. Also, the acyltransferase activity in serum of rats administered aspirin after inhibiting protein synthesis with cycloheximide was no longer elevated as compared with that of rats administered cycloheximide alone.

These results suggest that the elevation of the acyltransferase activity in serum of rats administered aspirin may not be due to the direct action of aspirin on the enzyme and the inductive action of aspirin on the protein synthesis.

Morris and Church [13] have reported that, when acyltransferase activity was expressed on the basis of percent of free cholesterol esterified, acyltransferase activity in serum of fasted rats is reduced, compared with that of control rats, while when acyltransferase activity is expressed as net esterification, fasting has no influence on acyltransferase activity. From these observations, they suggested that endogenous substrate concentration may be one of the most important factors in LCAT assay [13]. Therefore, we investigated the relationship between acyltransferase activity and free cholesterol content in serum of rats administered aspirin or salicylic acid. As shown in Table 1, free cholesterol content in serum of rats administered salicylic acid or aspirin was reduced to 73–78%, compared with that of control rats. Assuming that endogenous cholesterol in rat serum (12 mg protein/incubation medium) reached an equilibrium with the radioactive cholesterol (11.61 μg/incubation medium) in the sub-

strate solution during incubation, the amount of cholesterol esterified during incubation for 1 hr was 15.3 μg/ml serum (2.72 ± 0.01 μg/12 mg protein/hr) in control rat serum, 15.4 μg/ml serum (2.81 ± 0.06 μg/12 mg protein/hr) in aspirin-treated rat serum and 15.2 μg/ml serum (2.63 ± 0.11 μg/12 mg protein/hr) in salicylic acid-treated rat serum. A difference between the values for net esterification of cholesterol in serum of rats treated with and without the drugs was not observed. Furthermore, to clarify the relationship between acyltransferase activities and free cholesterol contents of a serum from control, starved and refed rats, the values were determined as reported by Morris and Church [13]. As shown in Table 1, when the acyltransferase activity was expressed as a percentage of free [^{3}H]-cholesterol esterified, the acyltransferase activities in the sera of starved and refed rats were higher than that of control rats. Assuming that endogenous cholesterol in rat serum reached equilibrium with radioactive cholesterol in the substrate solution during incubation as mentioned above, the amount of free cholesterol esterified during 1 hr of incubation was 15.6 μg/ml serum (2.46 ± 0.30 μg/12 mg protein/hr) in control rat serum, 14.2 μg/ml serum (2.46 ± 0.28 μg/12 mg protein/hr) in starved rat serum, and 15.4 μg/ml serum (2.65 ± 0.37 μg/12 mg protein/hr) in refed rat serum. A difference between the net esterification of cholesterol in starved and control rat sera could not be detected. Furthermore, the amounts of free and total cholesterol in serum of rats treated with and without the drugs were determined. As shown in Table 3, free cholesterol content in serum of rats administered aspirin or salicylic acid decreased markedly compared with that of control rats as well as with the results

Table 1. Relationship between LCAT activity and free cholesterol content in rat serum*

Treatment (50 mg/kg)	Cholesterol esterified (%)	Serum free cholesterol (μg/ml serum)	Net esterification (μg/ml serum/hr)
None (control)	14.4 ± 0.2 (100)	262.1 ± 6.7 (100)	15.3 ± 0.1 (100)
Aspirin	17.6 ± 0.7† (122)	203.9 ± 5.5‡ (78)	15.4 ± 0.8 (101)
Salicylic acid	18.4 ± 1.3 (128)	190.6 ± 7.9‡ (73)	15.2 ± 0.6 (99)
None (control)	14.1 ± 1.7 (100)	266.1 ± 3.3 (100)	15.6 ± 2.3 (100)
Starved	19.6 ± 2.0§ (139)	159.3 ± 16.3† (60)	14.2 ± 1.9 (91)
Refed	18.2 ± 1.0§ (129)	194.4 ± 8.0‡ (73)	15.4 ± 1.2 (99)

* Values are means ± S.E. of three experiments. The values in parentheses are percentages of the values obtained in control rat serum (taken as 100%).
† Significant at $P < 0.01$ by Student's *t*-test.
‡ Significant at $P < 0.001$ by Student's *t*-test.
§ Significant at $P < 0.05$ by Student's *t*-test.

Table 2. LCAT activity and free cholesterol content in serum of rats administered cycloheximide with or without aspirin *in vivo**

	Cholesterol esterified (%)		Serum free cholesterol (μg/ml serum)	
Treatment	Expt. I	Expt. II	Expt. I	Expt. II
None (control)	14.6 (100)	14.7 (100)	256.9 (100)	270.4 (100)
+ Cycloheximide	5.9 (40)	8.4 (56)	172.9 (67)	93.9 (35)
Aspirin	17.4 (119)	17.8 (121)	199.8 (78)	219.4 (81)
+ Cycloheximide	6.0 (41)	6.4 (44)	124.1 (48)	66.0 (24)

* Cycloheximide was injected intraperitoneally twice (8:00 p.m., 8:00 a.m.) a day for Experiment I and three times (8:00 p.m., 2:00 a.m., 8:00 p.m.) a day for Experiment II 1 hr prior to the administration of aspirin. The values in parentheses are percentages of the values obtained in control rat serum (taken as 100%).

Table 3. Free and total cholesterol content in serum of rats administered aspirin and salicylic acid *in vivo**

	Serum free cholesterol (μg/ml serum)			Serum total cholesterol (μg/ml serum)		
Treatment	Expt. I	Expt. II	Average	Expt. I	Expt. II	Average
None (control)	271.9	278.8	275.4 (100)	1051.9	1015.2	1033.6 (100)
Aspirin	217.9	231.1	224.5 (82)	972.0	936.2	954.1 (92)
Salicylic acid	199.0	184.4	191.7 (70)	873.8	754.5	814.2 (79)

* Values in parentheses are percentages of the values obtained in control rat serum (taken as 100%).

shown in Table 1. Similarly, total cholesterol content in serum of rats administered aspirin or salicylic acid was decreased to 79–92% of that of control rats. We have also found that the amounts of salicylic acid in the sera of rats administered aspirin and salicylic acid were 0.46 and 0.64 μmole/ml of serum respectively.

The elevation of the acyltransferase activity in serum of rats administered aspirin or salicylic acid orally seems to be due mainly to the decrease of serum free cholesterol content. In addition, the decrease of cholesterol content in rat serum by the administration of aspirin or salicylic acid may be due to the increase of excretion of cholesterol into bile, the increase of enzymatic conversion of cholesterol to bile acid, and/or the decrease of absorption of cholesterol from intestine. Experiments to clarify these problems are in progress now.

Faculty of Pharmaceutical Sciences
Kumamoto University
5-1 Ohe-Honmachi
Kumamoto 862, Japan

MITSUO NAKAGAWA*
REI TAKAHASHI
SHIORI JOHSAKI
TOSHIYA HONDA
MORIO KIYOZUMI
SHOJI KOJIMA

* Author to whom all correspondence should be addressed.

REFERENCES

1. J. A. Glomset, *J. Lipid Res.* **9**, 155 (1968).
2. S. N. Shah, W. J. Lossow and I. L. Chaikoff, *Biochim. biophys. Acta* **84**, 176 (1964).
3. J. A. Glomset, *Am. J. clin. Nutr.* **23**, 1129 (1970).
4. M. Nakagawa and T. Nishida, *J. Biochem., Tokyo* **74**, 1263 (1973).
5. S. E. Kramer and J. I. Routh, *Clin. Biochem.* **6**, 98 (1973).
6. K. D. Muirden, P. Deutschman and M. Phillips, *Aust. N. Z. J. Med.* **4**, 149 (1974).
7. D. Hawkins, R. N. Pimchard, I. P. Crawford and R. S. Farr, *J. clin. Invest.* **48**, 536 (1969).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
9. S. Batzri and E. D. Korn, *Biochim. biophys. Acta* **298**, 1015 (1973).
10. M. Nakagawa, M. Takamura and S. Kojima, *J. Biochem., Tokyo* **81**, 1011 (1977).
11. J. Folch, M. Less and G. H. Sloane-Stanley, *J. biol. Chem.* **226**, 497 (1967).
12. R. A. Muesing and T. Nishida, *Biochemistry* **10**, 2952 (1971).
13. R. S. Morris and J. P. Church, *Nutr. Rep. Int.* **5**, 407 (1972).

Biochemical Pharmacology, Vol. 33, No. 17, pp. 2817–2819, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

Similar potency ratios of amphetamine optical isomers for inhibition of dopamine uptake by synaptosomes of corpus striatum, olfactory tubercle and prefrontal cortex of the rat

(*Received* 15 *February* 1983; *accepted* 21 *February* 1984)

Although comparable doses of the optical isomers of amphetamine (AMPH) have been shown to produce or exacerbate psychotic symptoms in man [1–3], it has been shown in electrophysiological [4] and biochemical [5, 6] experiments that D-AMPH is much more potent than L-AMPH in its actions on dopamine (DA) neurons projecting to the corpus striatum from the substantia nigra (SN). The near equipotence of the isomers in their psychotogenic effects is thus difficult to interpret in the framework of the dopamine hypothesis of schizophrenia. However, recent evidence suggests that DA neurons of the ventral tegmental area (VTA) rather than those of the SN may be involved in schizophrenia [7–9]. In humans and in rats, VTA DA neurons innervate several limbic and cortical structures including nucleus accumbens, olfactory tubercle, prefrontal, cingulate and entorhinal cortex [10, 11]. Mesocortical DA neurons in particular are a unique subpopulation of DA neurons which appear to lack DA autoreceptors [12, 13]. Thus, mesocortical DA neurons, in contrast to mesolimbic and SN DA neurons, do not show decreases in rate of impulse flow or in tyrosine hydroxylase activity to low doses of the DA receptor agonist apomorphine. It is therefore possible that mesocortical DA neurons may have a unique response to other dopaminergic drugs, including the optical isomers of amphetamine. To test this possibility, we have established a method of selectively measuring DA uptake by DA nerve terminals and have tested the relative potencies of D- and L-AMPH on mesolimbic and mesocortical terminals as compared to nigrostriatal terminals. We have found that there is no difference in the potency ratios for the AMPH isomers in the three areas. D-AMPH is about 5-fold more potent than L-AMPH in each terminal region. A preliminary report of these findings has been published [14].

Materials and methods

Female Holtzman albino rats weighing 200–225 g were used in all experiments. Tissue preparation and uptake assays were done by a modified method of Horn *et al.* [15]. Briefly, rats were killed with chloroform asphyxiation, and brains were quickly removed and chilled in ice-cold saline. Samples of frontal cortex were taken as described by Bockaert *et al.* [16]. These consisted of a medial-dorsal wedge of cortex rostral to the nucleus accumbens. Samples of corpus striatum and olfactory tubercle were dissected bilaterally. Tissues were weighed and then homogenized with a Teflon pestle in glass tubes in 20 vol. of 250 mM sucrose. The homogenate was centrifuged at 1000 *g* for 10 min, and the supernatant fraction was used for incubations. Aliquots (100 μl) of the supernatant fraction were incubated with 1.9 ml of a modified Krebs–Henseleit buffer (95 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 4.9 mM pyruvate, 5.4 mM fumarate, 4.9 mM L-glutamate, 11.5 mM glucose, 12.5 μM nialamide, 0.2 mg/ml sodium EDTA, 0.2 mg/ml ascorbic acid) in a shaking water bath (37°) under a 5% CO_2/95% O_2 atmosphere. Drugs were added 5 min before [^{3}H]-DA (10^{-7} M). Incubations were terminated after 5 additional min by rapid filtration through 2.1 cm Whatman GF/C filters and washed twice with 5 ml of ice-cold buffer. Preliminary experiments showed linear accumulation of [^{3}H]-DA for at least 5 min in all three brain area preparations. Filters were extracted with toluene-base scintillation mixture for at least 30 min followed by estimation of tritium with a Beckman scintillation counter.

[^{3}H]-Dopamine (21.5 Ci/mmole) was obtained from New England Nuclear, AMPH isomers from Smith, Kline & French Laboratories, fluoxetine from Lilly Research Lab-