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ENZYMATIC HYDROLYSIS OF HALOPERIDOL DECANOATE AND ITS INHIBITION BY PROTEINS*

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Abstract—When [^{14}C]haloperidol decanoate, a long-acting neuroleptic and an ester of haloperidol and decanoic acid, was incubated in human whole blood and plasma and in rat plasma and homogenates of rat brain, lung, liver, kidney, pancreas and muscle, no hydrolysis of the ester was seen. Although the decanoate was hydrolyzed by partially purified carboxylesterase, addition of rat plasma or liver homogenate to the enzymic reaction mixture resulted in marked inhibition of hydrolysis, whereas addition of the defatted residues of plasma or liver produced only partial inhibition. The enzymic hydrolysis was inhibited also by β-lipoprotein and albumin, depending on their concentrations. The assumption that interaction between haloperidol decanoate and protein resulted in inhibition of the hydrolytic reaction mediated by the enzyme was validated by kinetic models and experimental data. The kinetics were apparently competitive. Based on the kinetic analysis, the interaction between the decanoate and albumin or β-lipoprotein was investigated by measuring their equilibrium constants and extent of protein binding. Haloperidol decanoate appeared to interact with several proteins; this was exemplified by other measures of protein binding, an increasing effect of proteins on the solubility, and the partition ratio of the ester. The interaction between haloperidol decanoate and proteins caused marked stabilization of this ester against enzymatic hydrolysis and, thereby, influenced its metabolism.

Haloperidol decanoate, an ester of haloperidol and decanoic acid, is a promising long-acting neuroleptic drug, used in psychiatry [1, 2]. Intramuscular injection of the ester dissolved in oil in an appropriate dose results in satisfactory clinical efficacy in patients, with sustained therapeutic plasma levels of haloperidol for periods up to 1 month. Our knowledge concerning the pharmacokinetic properties of the ester, however, is very limited. Particularly, the mechanism involved in the formation of the active principle of haloperidol from the ester has not been elucidated, similar to other depot antipsychotics of the ester type.

Recently, we found in rat studies‡ that the ester was rate-limiting for absorption via the lymphatic pathway, with the result that haloperidol remained in the plasma for a long period. In the course of disposition and metabolism studies of the ester, we found that the ester was rather stable in plasma and tissue homogenates against hydrolysis. Formation of the active principle of haloperidol was not demonstrated after incubation for a short time *in vitro*, seemingly similar to another long-acting neuroleptic, fluphenazine decanoate [3]. Although haloperidol decanoate was found to be readily hydrolyzed in the presence of partially-purified carboxylesterase (EC 3.1.1.1), enzymatic hydrolysis was inhibited markedly by addition of plasma and tissue homogenates to the reaction mixture. The present paper describes studies concerning the enzymatic formation of haloperidol from its decanoate and the inhibitory effect of biological materials on the formation.

MATERIALS AND METHODS

Chemicals. [*Carbonyl*-^{14}C]Haloperidol (specific radioactivity: 14.9 μCi/mg, 5.60 μCi/μmol), [*carbonyl*-^{14}C]haloperidol decanoate (specific radioactivity: 10.6 to 31.2 μCi/mg, 5.64 to 16.6 μCi/μmol) and [*carboxyl*-^{14}C]etofenamate, a highly lipophilic ester of flufenamic acid and diethyleneglycol [4] (specific radioactivity: 20.4 μCi/mg, 7.53 μCi/μmol) were prepared as described previously [5, 6]. Their radiochemical purities were >99%. Etofenamate was used as a reference compound for enzymic hydrolysis of haloperidol decanoate.

Unlabeled haloperidol decanoate and etofenamate were products of Janssen Pharmaceutica (Beerse, Belgium) and Troponwerke GmbH & Co., KG (Köln, West Germany) respectively. Haloperidol and flufenamic acid were supplied from the quality control unit of our factory. Type I and II carboxylesterases from porcine liver, type II lipase (EC 3.1.1.3) from porcine pancreas, and human serum albumin (fraction V) were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bovine β-lipoprotein was the product of the United States Biochemical Corp. (Cleveland, OH, U.S.A.).

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‡ Y. Matsunaga, K. Nambu, Y. Oh-e, H. Miyazaki and M. Hashimoto, manuscript submitted for publication.

All other chemicals of analytical reagent grade were obtained from various commercial sources.

Biological materials. Human blood was obtained by venipuncture from seven healthy male volunteers.

Male Wistar rats weighing about 250 g were decapitated. The brain, lung, liver, kidney, pancreas and femoral muscle were quickly excised, weighed, and homogenized in 9 vol. of ice-cold 0.1 M phosphate buffer (pH 7.4). Blood was collected in a heparinized syringe by puncture of the abdominal artery in other rats under light ether anesthesia and was centrifuged for separation of plasma. For the preparation of defatted materials, both plasma and liver homogenates were added to 2 vol. of ethanol and centrifuged. The residues were each extracted six times with 5 vol. of an ethanol/ether mixture (2/1, v/v) and then with ether on repeated centrifugations. The defatted residues were dried under reduced pressure, resuspended in the same volume of the buffer as the starting homogenate, and used as an additive to the enzyme reaction mixture.

Incubation. All of the following incubations were done in well-rinsed clean glassware (usually glass test tubes) because labeled esters in aqueous solution were found to be adsorbed extensively onto the surface of the glass. Only the use of glassware made it possible to get experimental data with sufficient reproducibility; this phenomenon will be discussed in detail in the next section.

For examination of hydrolysis of haloperidol decanoate in biological materials, a 1-ml portion of human blood diluted to 71% with physiological saline, of human plasma diluted to 71% with the buffer, of 10% rat plasma in the buffer, or of 10% rat tissue homogenates was mixed with less than or equal to 10 μl of a known concentration of an ethanolic solution of [^{14}C]haloperidol decanoate or [^{14}C]etofenamate and incubated at 37° for 1.5 hr. Other experimental conditions are indicated in the footnote to Table 1.

For examination of enzymic hydrolysis of the esters, the reaction mixture contained less than or equal to 10 μl of ethanolic solution of a labeled ester and approximately 500 μg (type I) or 1 mg (type II) of the carboxylesterase preparation or about 5 mg of a lipase preparation, per 1.0 or 1.2 ml of the phosphate buffer, pH 7.4, respectively. Other experimental conditions are presented in the footnotes to the respective experimental results (Tables 2 and 3, Fig. 1). In the studies of the kinetics of the enzyme reactions, the concentrations of labeled esters were determined by actual measurements of the radioactivities of the reaction mixtures because of the extensive adsorption of the esters onto the surface of the glassware, as mentioned above.

All incubations were terminated by the addition of 2 vol. of ethanol to the incubation mixtures.

Protein binding. For estimation of protein binding of haloperidol decanoate by the method of adsorption change (described in the next section), [^{14}C]haloperidol decanoate and protein were incubated for 30 min at 37°. By the method of partition change [7], *n*-heptane that contained the labeled ester was gently shaken for 48 hr at 4° with the aqueous buffer containing approximately physiological concentrations of proteins. The concentration of labeled ester was determined by actual measurement of radioactivity.

Analyses. After the addition of ethanol to the incubation mixtures, the deproteinized supernatant fraction was analyzed for hydrolysis of [^{14}C]haloperidol decanoate and [^{14}C]etofenamate by thin-layer radiochromatography [8, 9]. Developed plates were scanned by a radiochromatogram scanner (Packard Instruments, Inc., IL, U.S.A.), and the radioactive components (unchanged ester and hydrolysate, [^{14}C]haloperidol or [^{14}C]flufenamic acid) on the resultant radiochromatogram were quantified by their peak areas. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer model 3380 with quenching correction by external gamma as previously described [8]. The radioactivity determined was converted to nanomoles of the compound based on its specific radioactivity.

RESULTS AND DISCUSSION

Hydrolysis of haloperidol decanoate in human plasma and blood and in rat plasma and tissue homogenates. After [^{14}C]haloperidol decanoate was incubated for 1.5 hr in human blood, human and rat plasma, and homogenates of rat brain, lung, liver, kidney, pancreas and muscle, no hydrolysis of the ester was seen. In contrast, another lipophilic ester, [^{14}C]etofenamate, which is stable against spontaneous degradation under these conditions [9], was hydrolyzed to produce [^{14}C]flufenamic acid to various extents. A typical result in a series of these experiments is presented in Table 1. The finding that hydrolysis of haloperidol decanoate was not detected after a 1.5-hr incubation is similar to observations with fluphenazine esters [3, 10], but it is in striking contrast to findings with other long-acting neuroleptic esters, e.g. decanoates of flupenthixol [11] and clopenthixol [12], which are reported to be easily hydrolyzed under similar conditions. The fact that haloperidol decanoate was quite stable in blood and plasma may be related to the safety of this ester; it suggests that haloperidol, the active neuroleptic, will not be produced within a short period in blood into which the ester, though it is to be injected intramuscularly as a depot preparation [2], can be accidentally introduced directly, for instance, by an inadequate injection or by injury of the administered depot site. On the other hand, a problem has arisen concerning the marked stability of the ester in biological systems which is also a common problem for depot neuroleptics [13]. The present experiments are an attempt to understand the problem.

Enzymatic hydrolysis of haloperidol decanoate and its binding by proteins. [^{14}C]Haloperidol decanoate was not hydrolyzed when incubated with a lipase preparation. The decanoate and [^{14}C]etofenamate, however, were hydrolyzed by partially purified carboxylesterase preparations of porcine liver. The following studies were performed with the type I carboxylesterase preparation, and the cited data are typical of the experiment, based on a series of repeated examinations.

When rat plasma or liver homogenate was added to the enzyme reaction mixture, the hydrolysis of

Table 1. Hydrolysis of [^{14}C]haloperidol decanoate and [^{14}C]etofenamate *in vitro*

Animal	Tissue	Hydrolysis (%)	
		Haloperidol decanoate	Etofenamate
Human	Plasma	ND	—
	Whole blood	ND	90
Rat	Plasma	ND	8
	Brain	ND	ND
	Lung	ND	61
	Liver	ND	92
	Kidney	ND	100
	Pancreas	ND	—
	Muscle	ND	T

[^{14}C]Haloperidol decanoate (human, 32–108 nM; rat, 145 nM) or [^{14}C]etofenamate (human, 194 nM; rat, 136 nM) was incubated in 71% (human) diluted specimens or 10% (rat) plasma and tissue homogenates for 1.5 hr at 37°. Values are means of three to four humans and two rats. Abbreviations: (ND) not detected; (T) <1%; and (—) not determined.

Table 2. Effects of added materials on the hydrolysis of [^{14}C]haloperidol decanoate and [^{14}C]etofenamate by carboxylesterase from porcine liver

Additive	Hydrolysis (%)	
	Haloperidol decanoate	Etofenamate
None	79	100
Ethanol*	ND	—
Plasma	ND	100
Liver homogenate	ND	100
Plasma residue defatted	46	100
Liver residue defatted	29	100

[^{14}C]Haloperidol decanoate (145 nM) and [^{14}C]etofenamate (136 nM) were incubated with 500 μg of type I carboxylesterase preparation for 1.0 hr at 37° in 1 ml of 0.1 M phosphate buffer (pH 7.4) containing a final concentration of 10% of indicated biological materials and 10 μl of ethanolic solution of the labeled esters. Abbreviations: (ND) not detected; and (—) not determined.

* Two volumes of ethanol was added before incubation.

haloperidol decanoate ceased, whereas that of etofenamate continued (Table 2). These results suggest that some inhibiting material(s) acted on the substrate rather than on the enzyme under these conditions. Because the ester is highly lipophilic, the experiment was repeated using defatted residues of plasma and liver homogenates. In this case, a partial, but not a complete, inhibition of hydrolysis was seen, suggesting that certain lipophilic and/or ethanol-precipitable materials may have inhibited the reaction. The effects of known biological materials on the reaction were then examined.

The hydrolysis of haloperidol decanoate by esterase was diminished markedly by β-lipoprotein and, to a lesser extent, by serum albumin in a concentration-dependent manner (Table 3). Hydrolysis of etofenamate was not affected by these proteins. Albumin that had been preincubated with palmitate eliminated the hydrolysis of haloperidol decanoate completely, whereas the same concentration of albumin without added palmitate diminished the reaction only partially. These results suggest that macromolecules with certain lipophilic substituents diminished hydrolysis more significantly when the substrate was haloperidol decanoate, because of the interaction of this ester with proteins of a highly hydrophobic nature.

The fact that haloperidol decanoate interacted with proteins that protected this ester from enzymatic hydrolysis is conceivably the cause of the apparent lack of hydrolysis of the ester in plasma and tissue homogenates (Table 1). As pointed out before, fluphenazine decanoate [3] is similar to haloperidol decanoate in that it is not easily hydrolyzed in plasma or tissue homogenates within a short incubation time. In contrast, decanoates of flupenthixol [11] and clopenthixol [12] are readily hydrolyzed in such biological preparations. Haloperidol [14] and fluphenazine [15] have higher partition ratios ($> 10^3$) between *n*-octanol/aqueous solvents than flupenthixol and clopenthixol (approximately 2×10^2) [15]. Therefore, it seems that hydrophobic interactions

Table 3. Effect of added materials on the hydrolysis of [^{14}C]haloperidol decanoate and [^{14}C]etofenamate by carboxylesterase from porcine liver

Additive	Hydrolysis (%)	
	Haloperidol decanoate	Etofenamate
None	62	100
β-Lipoprotein		
0.6 mg/ml	12	—
3 mg/ml	4	—
16 mg/ml	ND	100
Albumin		
8 mg/ml	66	—
40 mg/ml	29	—
200 mg/ml	11	100
Albumin-Palmitate*	ND	—

[^{14}C]Haloperidol decanoate (145 nM) and [^{14}C]etofenamate (136 nM) were incubated with 500 μg of type I carboxylesterase preparation with indicated concentrations of additives in 1 ml of phosphate buffer (pH 7.4) for 1.5 hr at 37°. Abbreviations: (ND) not detected; and (—) not determined.

* A saturated concentration of sodium palmitate in buffer was preincubated with 40 mg/ml of albumin for 30 min at 37°.

between these long-acting neuroleptics of the ester type and protein are influenced not only by their decanoic acid chain but also greatly by their alcoholic moiety and, therefore, affects the metabolism of the esters. The protection afforded by proteins against enzyme attack of the substrate is first demonstrated in this paper, although stabilization of a substance by its interaction with protein against its spontaneous degradation has been reported: e.g. for endogenous leukotriene [16] and exogenous melphalan [17], and others [18].

Mode of inhibition. As suggested above, possible interaction between haloperidol decanoate and proteins resulted in the apparent inhibition of the hydrolytic reaction mediated by carboxylesterase, the validity of which should be examined. Assuming this is true, the substrate, carboxylesterase, protein, and the product formed by the enzyme reaction are abbreviated as *S, E, p* and *P,* respectively, and the following reaction scheme is described:

$$\begin{array}{l} S + E \leftrightharpoons ES \xrightarrow{K} P + E \\ + \\ p \\ \Updownarrow \\ Sp \end{array}$$

Here, *ES, k* and *Sp* are enzyme–substrate complex, the rate constant of *ES* dissociation into *P* and *E*, and substrate–protein complex respectively. The conservative equations are

$$[S_0] = [S] + [ES] + [Sp] + [P] \quad (1)$$

$$[E_0] = [E] + [ES] \quad (2)$$

$[S_0]$ and $[E_0]$ are the initial concentrations of *S* and *E* respectively. When the dissociation constant of *S* and *p* is expressed as K_d,

$$K_d = [S][p]/[Sp], [Sp] = 1/K_d \times [S][p] \quad (3)$$

and that of *S* and *E*, as K_s,

$$K_s = [E][S]/[ES], [ES] = 1/K_s \times [E][S] \quad (4)$$

The reaction rate,

$$v \simeq k[ES] = [P], [P] = k/K_s \times [E][S] \quad (5)$$

From $[S] \gg [E]$, and from Equations 3, 4, and 5, the conservative Equation 1 can be converted to

$$[S] = [S_0]/(1 + [p]/K_d) \quad (6)$$

Since $V_{max} = k[E_0]$, with Equations 2, 4 and 6, we obtain

$$v/V_{max} = [S]/(K_s + [S])$$
$$= [S_0]/\{K_s(1 + [p]/K_d) + [S_0]\} \quad (7)$$

This equation is apparently the same as that given for the usual competitive enzyme inhibition: $v/V_{max} = [S]/\{K_s(1 + [I]/K_i) + [S]\}$, where $[I]$ and K_i are the concentration of the inhibitor and the dissociation constant of the inhibitor and enzyme respectively. Therefore, the inhibition of hydrolysis of haloperidol decanoate in the presence of a protein would apparently be of the competitive type. In fact, the kinetics of the reaction was competitive as shown in double-reciprocal plots (Fig. 1a) when the inhibitor, albumin, was present. This finding supports the validity of the assumption that interaction between *S* and *p* resulted in a diminution of the enzymatic hydrolysis of haloperidol decanoate (*S*). In the absence of albumin, K_s, (K_m of the esterase for haloperidol decanoate) and V_{max} were 2.70 μM and 52.1 nM/min, respectively, under the conditions employed.

It is generally recognized that competitive inhibition is derived from competition between substrate and inhibitor for the active site of the enzyme. In the case of haloperidol decanoate, however, the esterase and protein appeared to compete for the substrate. The dissociation constant, K_i, is the equilibrium constant between the inhibitor and enzyme in the former case, but in the latter case, K_d, between the apparent

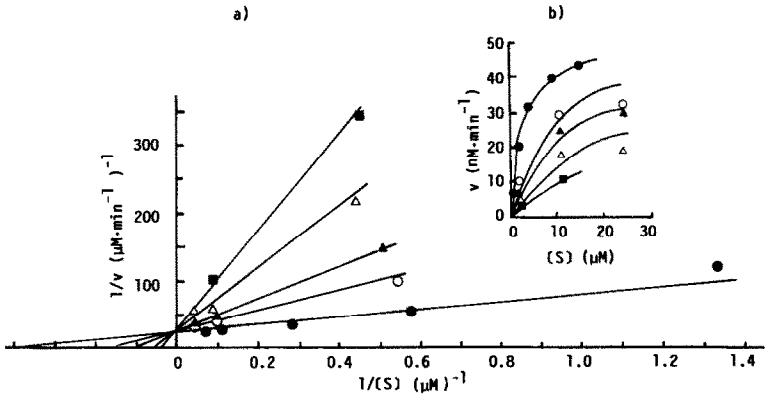

Fig. 1. Effect of albumin on the hydrolysis of [^{14}C]haloperidol decanoate by carboxylesterase. Key: (a) Lineweaver–Burk plot, and (b) Michaelis–Menten plot used for determination of protein binding; see text. Albumin concentrations: 0 μM (●), 75.8 μM (○), 152 μM (▲), 303 μM (△), and 606 μM (■). [^{14}C]Haloperidol decanoate and type I carboxylesterase preparation (250 μg) were incubated in 0.5 ml of 0.1 M phosphate buffer (pH 7.4) at 37° for 30 min. The concentration of the labeled ester [S] was that actually determined.

inhibitor (protein) and the substrate (haloperidol decanoate). Therefore, the dissociation constant of protein binding of haloperidol decanoate can be calculated using Equation 7. That is, at a concentration of the inhibitor protein, the Michaelis constant K_m is obtainable, since usual Michaelis–Menten kinetics gives the equation $v/V_{max} = [S]/(K_m + [S])$, from the equation $K_m = K_s(1 + [p]/K_d)$. Under these conditions, the K_m is a function of $[p]$ as shown by

$$K_m = K_s/K_d \times [p] + K_s \qquad (8)$$

For determination of K_m at the examined concentration of albumin, the Michaelis–Menten equation was converted to $K_m = [S](V_{max}/v - 1)$, based on which K_m values were calculated as presented in Table 4. From these K_m and $[p]$ values, the linear regression equation of (8) was $K_m = 0.0657[p] + 2.70$ ($|r| = 0.972$). From this, $K_d = 4.11 \times 10^{-6}$ M was obtained. The association constant between haloperidol decanoate and albumin, $K_a = 1/K_d = 2.43 \times 10^5\ M^{-1}$.

Using β-lipoprotein at concentrations of 1.25 to 5.0 μM as an inhibitor, similar experiments were performed. The protein also exhibited apparently competitive inhibition of the hydrolysis of haloperidol decanoate by the carboxylesterase. The dissociation constant K_d and association constant K_a between β-lipoprotein and haloperidol decanoate were calculated as described above (Table 4) to be 1.74×10^{-7} M and $5.75 \times 10^6\ M^{-1}$ respectively.

Binding of haloperidol decanoate to protein. As described above, the interaction between haloperidol decanoate and protein seems to be an important factor in the metabolism of this ester to produce active principle haloperidol and hence in the pharmacology of this prodrug, since the ester is not centrally active [19] nor does it penetrate the brain.*

Table 4. Concentrations of protein and substrate, and reaction rate and derived K_m values

Protein	Concentration (μM) [p]	Concentration (μM) [S]	v (nM/min)	K_m (μM)
Albumin	75.8	1.85	10.2	7.58
		10.2	29.3	7.92
		24.0	31.9	15.1*
	152	1.97	6.97	12.7
		10.5	24.7	11.6
		24.3	29.4	18.6
	303	2.26	4.63	23.1
		11.3	17.7	22.0
		24.0	18.4	43.7*
	606	2.22	2.93	37.2
		11.2	10.1	46.7
β-Lipoprotein	1.25	2.36	6.17	15.4
		11.1	20.6	13.8
	2.50	2.49	3.06	35.3
		12.4	14.1	28.3
		25.2	18.9	36.7
	5.00	2.55	1.49	76.7
		12.5	6.53	76.1
		30.3	12.7	80.2

Abbreviations: $[p]$, concentration of protein; $[S]$, actual concentration of [^{14}C]haloperidol decanoate; v, concentration of [^{14}C]haloperidol formed per 1 min = $[P]$. For calculation of K_m values see text.

* Omitted for the calculation of linear regression.

It also suggests that the interaction of depot antipsychotic drugs with hydrophobic materials is the basis of their stability at the depot site and in plasma and tissues. Therefore, attempts were made to elucidate the interaction between haloperidol decanoate and proteins mainly by the extent of protein binding. Determination of protein binding of haloperidol decanoate by convenient physical means such as

* Y. Matsunaga, K. Nambu, Y. Oh-e, H. Miyazaki and M. Hashimoto, manuscript submitted for publication.

ultrafiltration using Amicon ultrafiltration membranes, equilibrium dialysis with celophan membrane, and gel filtration by Sephadex were inadequate for the ester since it was adsorbed completely onto the materials of filtration at a wide range of concentrations. On the other hand, as seen in Fig. 1b, measurement of the extent of binding of haloperidol decanoate to protein, in addition to previously determined equilibrium constants K_d and K_a, can be expressed alternatively by the degree of enzyme inhibition, since we assumed that only free (unbound to protein) substrate can participate in the reaction. That is, at a given reaction rate, the concentration of haloperidol decanoate in the presence of an inhibitor protein would be the sum of the concentrations of free and bound fractions (S_t) of the substrate (decanoate), and the concentration of the substrate in the absence of protein should be equal to that of the free fraction (S_f). Therefore, the extent of binding of the substrate to the protein can be expressed by the equation, binding $(\%) = (S_t - S_f)/S_t \times 100$. Using this, the extent of binding of haloperidol decanoate to human serum albumin was estimated graphically in Fig. 1b at an S_t near the observable plasma levels in rats.* Results are given in Table 5, where the extent of binding to β-lipoprotein similarly calculated is also listed.

As briefly stated in the experimental portion of this paper, addition of a small volume of [^{14}C]haloperidol decanoate dissolved in ethanol into aqueous buffer solution resulted in extensive adsorption of the ester onto the surface of the glassware and/or in phase separation between water and oil (the ester), depending on the concentration. Therefore, it was impossible to prepare a known concentration of the aqueous solution of the ester by calculation; actual measurement of the radioactivity of the resulting aqueous solution was necessary to know the actual concentration of the ester. In addition, it was also found that the actual concentration of the ester was lower in plain buffer (free of protein) than with added protein, when the same amount of ester was added to the buffer: the protein apparently increased the solubility of haloperidol decanoate. The effects of human serum albumin and β-lipoprotein on the solubilization of [^{14}C]haloperidol decanoate are illustrated in Fig. 2 and are partly similar to the effect of a detergent of Bs type [20]. The effect of proteins can be regarded as derived from the interaction between the ester and the protein, which is reflected by an increment in the solubility between zero and a given protein concentration; the extent of binding of the ester to protein was estimated as listed in Table 5. The increasing effect of protein on the solubility of a substance has been demonstrated with phenytoin, from which it was inferred that the phenomenon was based on the formation of a metastable complex of the drug and protein [21].

Another approach was also used to elucidate the interaction between the ester and protein, by observing the change in partition of the ester between *n*-heptane and the buffer, with and without protein in the aqueous buffer phase [7]. A typical regression

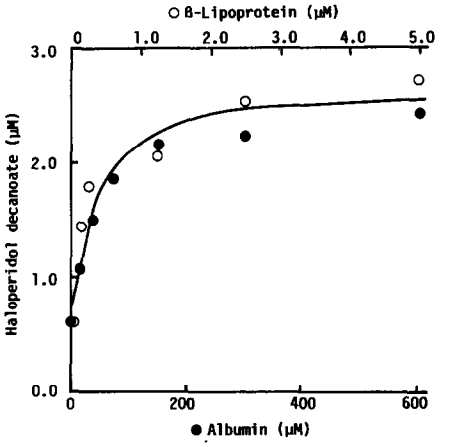

Fig. 2. Effects of albumin and β-lipoprotein on the solubility of [^{14}C]haloperidol decanoate in the aqueous buffer. A known amount of [^{14}C]haloperidol decanoate and various concentrations of proteins [(●) albumin, and (○) β-lipoprotein] were incubated in 0.1 M phosphate buffer (pH 7.4) for 30 min at 37°, and the concentration of the labeled ester in the buffer was actually determined. The theoretical concentration of [^{14}C]haloperidol decanoate was 2.83 μM in all systems.

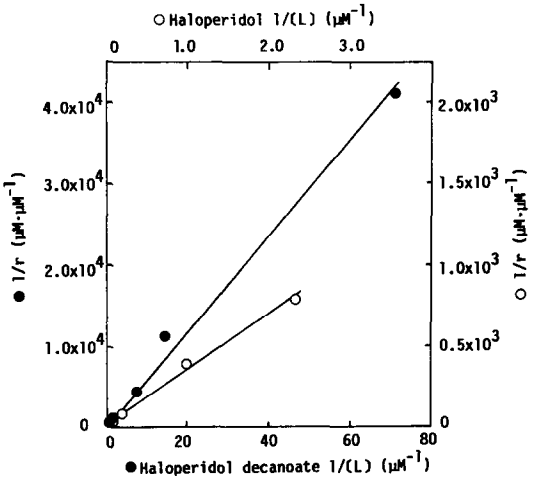

Fig. 3. Double-reciprocal plots of the binding of [^{14}C]haloperidol decanoate and [^{14}C]haloperidol to human serum albumin. *n*-Heptane containing the labeled compounds was shaken gently for 48 hr at 4° with 0.1 M phosphate buffer (pH 7.4) containing a physiological concentration of albumin. The concentrations of the labeled compounds were determined from these radioactivities. [*L*] is the concentration of free ligand and *r* is the binding ratio of the bound ligand to the total protein. The plotting of data and the criteria for linear regression were based on Ref. 7.

line, with an intersection point at the origin (Fig. 3), was obtained for the interaction between human serum albumin and [^{14}C]haloperidol decanoate or [^{14}C]haloperidol (as a reference compound), demonstrating the interaction of low affinity and hydrophobicity with infinite binding sites [7]. From this, the extent of protein binding was calculated (Table 5). Determination of the interaction between the ester and β-lipoprotein was not done because gradual

* Y. Matsunaga, K. Nambu, Y. Oh-e, H. Miyazaki and M. Hashimoto, manuscript submitted for publication.

Table 5. Protein binding of [^{14}C]haloperidol decanoate measured by different methods

Method	Protein	Concentration (ng/ml)		Protein binding (%)
		Total	Free drug	
Esterase inhibition	Albumin	100	8*	92
	β-Lipoprotein	100	24*	76
Solubilization	Albumin	1300	316	76
	β-Lipoprotein	1100	316	71
Partition	Albumin	16	7	55
		69	36	48
		153	70	54
		100†	19	81

Concentrations of albumin and β-lipoprotein were their physiological levels in serum.
* Ratio of free drug/total drug: see text.
† [^{14}C]Haloperidol, as a reference.

denaturation of the protein seemed to occur under the conditions employed.

Starting from the findings on the marked stability of haloperidol decanoate in plasma and tissue homogenate, the present work has revealed that the hydrophobic interaction between the ester and proteins was the cause of the stability. As already implied in this paper, the stability may possibly play a role in the safety of this ester if accidentally present in the blood because the potent parent drug haloperidol would not be released within a short time. In addition, the ester *per se* is pharmacologically inactive [19] and, therefore, to obtain neuroleptic activity, its hydrolysis must occur. The degree of strength of the interaction can only be qualitatively estimated from the fact that, differing from haloperidol decanoate, enzymatic hydrolysis of etofenamate (a highly lipophilic ester that conceivably interacts with proteins) was not influenced at all by protein. A comparison of partition ratios of haloperidol [14] with those reported [15] for fluphenazine, flupenthixol and clopenthixol suggests that the physico-chemical properties of the alcoholic moiety (active principle) of long-acting neuroleptic esters are related to the interaction with protein and thereby with its metabolism. The interaction of haloperidol ester and protein that resulted in the inhibition of the carboxylesterase-mediated reaction was validated and quantitatively elucidated by the kinetics of the enzyme reaction. Furthermore, the multiplicity of the interactions between haloperidol decanoate and proteins can be alternatively characterized by determination of protein binding by two other methods.

Since *in vitro* hydrolysis of haloperidol decanoate did not occur easily in human blood and rat plasma and was inhibited completely by the plasma even in the presence of carboxylesterase, the ester would be completely bound to a variety of proteins such as albumin and lipoproteins in the plasma and would not be hydrolyzed to haloperidol in circulating blood or, probably, in extracellular fluid, within a short time period. Haloperidol decanoate was taken up rather efficiently and hydrolyzed* intracellularly to various extents by isolated rat liver cells, isolated human and rat lymphocytes of the primary culture, and cultured cells of established lines. The fate of haloperidol decanoate present in rat plasma†, therefore, which would be derived from the unhydrolyzed fraction of the ester during its lymphatic absorption†, should be directed toward distribution to tissue cells rather than hydrolysis there. In fact, after intramuscular administration of haloperidol decanoate in rats, the ester together with its hydrolysate haloperidol was found [5] in tissues such as the liver and kidney as well as in plasma.

The present work has revealed that binding of haloperidol decanoate to proteins is an important factor in its disposition and metabolism and in its safety and pharmacology. Besides, it provided a type of inhibition of enzymic reaction which was caused by an apparent competition for the substrate by the enzyme and the binding protein. Such mechanisms, that may be involved in the metabolism of haloperidol decanoate, conceivably may occur with depot antipsychotic drugs.

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