Selective Inhibition of Bitter Taste of Various Drugs by Lipoprotein

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Previously, we demonstrated that lipoprotein composed of phosphatidic acid (PA) and β-lactoglobulin (LG) selectively and reversibly suppress the frog taste nerve response to bitter substances. In the present study, we examined the effects of various lipoproteins on the taste sensation to various stimuli in humans by a psychophysical method. Among various lipoproteins composed of different of lipids and proteins, the lipoproteins composed of PA and proteins were most effective in suppressing bitter taste. The lipoproteins composed of PA and LG, bovine serum albumin, ovalbumin, α-lactoalbumin or casein similarly suppressed effects on sensation of bitter taste. Using PA-LG, the effects on taste sensation to various stimuli were examined. The bitter taste of all twelve substances examined was inhibited, while saltiness of NaCl and sweetness of sucrose were not inhibited. The inhibition of bitter taste was completely reversible. Masking of the target sites for bitter substances on the taste receptor membranes with PA-LG seems to contribute to the inhibition of bitter taste. Direct binding of the bitter substances to PA-LG in the medium also contributes to the inhibition of bitter taste of certain substances. Among various drugs, basic and hydrophobic substances such as quinine, denatonium and propranolol have low taste thresholds and are said to be the most bitter. PA-LG most effectively suppressed the bitter taste of such substances. PA originates from soybeans and the proteins used except for bovine serum albumin originate from milk or eggs, and hence the lipoproteins can be safely used to mask the bitter taste of drugs.

KEY WORDS: lipoprotein; alkaloids; bitter taste; taste inhibition; phosphatidic acid; β-lactoglobulin.

INTRODUCTION

Many drugs and in particular, alkaloids carrying a positive charge at a neutral pH elicit a strong bitter taste (1). Drug coating with sugar or polymers (2-4) or chemical modification of the drugs into insoluble derivatives (5) have been applied to mask bitterness. These methods are not always applicable, and new methods to mask the bitter taste of drugs are still required.

In general, bitter substances are hydrophobic, and thus hydrophobic interaction of the substances with the receptor sites contributes greatly to their binding (6-8). In previous studies (9, 10), we found that the lipoprotein composed of phosphatidic acid (PA) and β-lactoglobulin (LG) inhibits the frog taste nerve responses to the bitter substances without affecting those to sugars, amino acids, salts or acids.

In the present study, we examined the effects of lipoproteins composed of various species of proteins and lipids on human taste sensation. Lipoproteins composed of PA and the proteins inhibited bitter tastes of various substances without inhibiting the saltiness of NaCl or sweetness of sucrose. PA and the proteins used except for bovine serum albumin originate from foods such as soybean, milk and eggs, and hence the lipoproteins can be used safely to mask the bitter taste of drugs.

MATERIALS AND METHODS

PA was prepared by allowing the reaction of phospholipase D (Asahi Chemical Industry Co., Tokyo) with soybean phosphatidylcholine (Lucas Meyer, Hamburg) in a solvent composed of *n*-hexane, ethyl acetate and 100 mM sodium acetate buffer (pH 8.0) containing 450 mM $CaCl_2$ (1:0.5:1) according to the method of Yang *et al.* (11) with slight modifications. LG was purchased from Sigma Chem. Co., St. Louis.

Several methods of preparing phospholipid-protein complexes have been published (12-15), in which mixtures of phospholipid and protein are sonicated. In the present study, we found that lyophilization instead of sonication also caused the formation of phospholipid-protein complexes. For example, PA-LG was prepared as follows. Two grams of PA and 5 g of LG were suspended in 50 ml of water. The suspension was homogenized with a Polytron (Kinematica GmbH, Littau, Switzerland) and the resultant homogenate was freeze-dried. Extraction of the lyophilized powder with *n*-hexane, which is a good solvent for PA, transferred only 5% of the PA to the solvent, suggesting that PA in the powder is mostly bound to LG. The powder was easily dispersed in water, yielding a transparent solution with only a slight turbidity. Elution profile of PA-LG from a Sephacryl column showed a single peak at molecular weight of 500,000-1000,000. Detailed physicochemical properties of PA-LG will be published in a separate paper. Other complexes were prepared by a similar method to that employed for preparation of PA-LG. PA liposome (multilamellar vesicle) was prepared as follows. PA chloroform solution was poured into a flask and evaporated to dryness. Water was then added to the flask, and the mixture was agitated with a Vortex mixer.

The sources of bitter substances were as follows; quinine hydrochloride, caffeine monohydrate, *L*-phenylalanine, promethazine hydrochloride, propranolol hydrochloride, berberine chloride, theophylline, strychnine nitrate, brucine dihydrate and glycyl-*L*-leucine from Wako Pure Chem. Ind. Ltd., Osaka; naringin from Nacalai Tesque, Kyoto; denatonium benzoate from Sigma Chem. Co., St. Louis. Sources of other chemicals were as follows; soybean phosphatidylcholine (PC) from Lucas Meyer, Hamburg; soybean triacylglycerol (TG) from Nissin Oil Milles, Tokyo; casein, α-lactoalbumin (LA), bovine serum albumin (BSA), LG and ovalbumin (OVA) from Sigma Chem. Co., St. Louis. Diacylglycerol (DG) composed of glycerol and fatty acid from rapeseed produced by enzymatic reaction was supplied by Kao Corp., Kashima.

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Abbreviations: BSA, bovine serum albumin; DG, diacylglycerol; LA, α-lactoalbumin; LG, β-lactoglobulin; OVA, ovalbumin; PA, phosphatidic acid; PC, phosphatidylcholine; TG, triacylglycerol.

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Evaluation of Binding of Bitter Substances to PA-LG in Medium

Bitter substances were dissolved in 3.0% PA-LG solution and the resultant solutions were filtered with M.W. 5000 cut filters by centrifugation (3000 rpm x 20 min, KR-1500, Kubota, Tokyo). The quantity of the bitter substance in the filtrate was determined by reverse phase chromatography using a Li-Chrospher 100 RP18 (5 μm) column (250 mm x 4 mm, Merk) and elution solvent (MeOH/sodium pentanesulfonate, MeOH/phosphate buffer, pH 7.0, or CH_3CN/trifluoroacetic acid). The flow rate was 1 ml/min or 1.2 ml/min.

Evaluation of Effects of Lipoproteins on Human Taste Sensation

The effects of the lipoproteins on human taste sensation were psychophysically evaluated according to the method employed previously (16). The subjects were paid volunteers drawn mainly from students of the Faculty of Pharmaceutical Sciences and staff of Kao corporation, Kashima Research Laboratories. Six to twelve subjects were participated in each experiment.

The test solutions were prepared by dissolving chemicals in deionized water or solutions containing PA-LG of various concentrations. The pH of the stimulus solutions was 5-7. Standard solutions were prepared by dissolving quinine sulfate, NaCl and sucrose of different concentrations in deionized water.

About 5 ml of each solution was added to test tubes and presented at room temperature. The subjects were required to compare the taste intensity of a test solution with those of the standard solutions and to select a standard solution with a taste intensity equivalent to that of the given test solution. The subjects were instructed to scoop a teaspoonful of solution, to place the solution on the tongue, to taste it and to rinse their mouths thoroughly with deionized water after tasting each solution. The effects of lipoproteins on taste sensation were expressed by R (normalized concentration of standard solution the taste intensity of which is equivalent to that of the test solution), defined as follows;

$$R = \Sigma R_i n_j / \Sigma n_j \quad (1)$$

Here, R_i is the concentration of a normalized standard solution; $R_i = C_i/C_o$ where C_i is the concentration of the standard solution whose taste intensity is equivalent to that of the test solution and C_o is the concentration of standard solution the taste intensity of which is equivalent to that of the test solution containing no lipoprotein. n_j is the number of subjects.

RESULTS AND DISCUSSION

We prepared lipoproteins composed of LG and various lipids such as PA, PC, TG and DG, and examined their effects on the bitterness of 0.5 mM quinine. The effects were evaluated by the psychophysical method. Figure 1 shows the effects of the lipoproteins as a function of their concentrations, where relative bitterness is expressed by the normalized concentration (R) of the standard bitter solution with equivalent bitterness to the test solution. The bitterness decreased with increasing PA-LG concentration (Fig 1). On the other hand, lipoproteins of LG with other lipids only partly suppressed the bitterness. Thus, PA-LG was the most effective in suppressing bitterness of quinine among the lipoproteins examined.

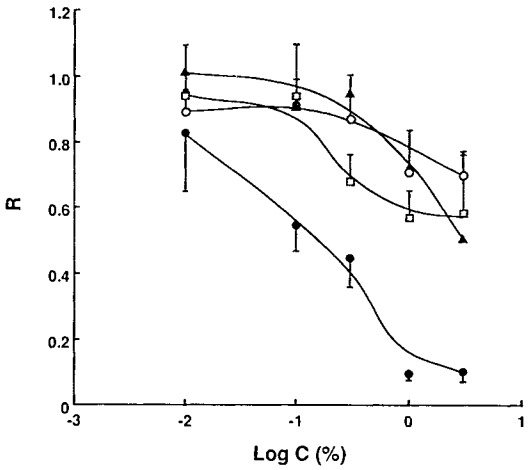

Figure 1. Effects of various lipoproteins on the bitter taste of 0.5 mM quinine as a function of their concentration. The relative taste sensation (R) was defined in Materials and Methods. Each point represents R ± SE obtained from 8-10 volunteers. ○, TG-LG; ▲, DG-LG; □, PC-LG, ●, PA-LG

Figure 2 shows the effects of PA-LG on the bitter taste of 5 mM promethazine, 10 mM propranolol and 50 mM caffeine, salty taste of 400 mM NaCl and sweet taste of 600 mM sucrose as a function of PA-LG concentrations. The bitterness of promethazine and propranolol was decreased to nearly zero at 3.0%, while that of caffeine was decreased to the 0.42 level at 3.0%. The effects of PA-LG were completely reversible since washing of the tongue with water after tasting solutions containing PA-LG and the bitter substances immediately recovered the function to taste their bitterness (data not shown). On the other hand, saltiness and sweetness were practically unchanged by PA-LG below 1.0% and

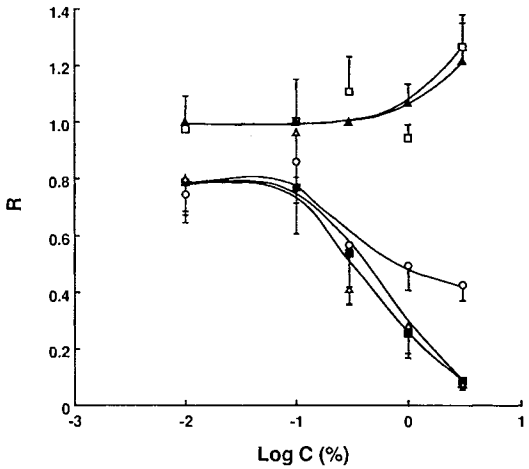

Figure 2. Effects of PA-LG of various concentrations on 400 mM NaCl (▲), sweetness of 600 mM sucrose (□) and bitterness of 10 mM propranolol (△), 5 mM promethazine (■) and 50 mM caffeine (○). The relative taste sensation (R) was defined in Materials and Methods. Each point represents R ± SE obtained from 8-10 volunteers.

slightly increased with further increases in PA-LG concentration.

The effects of lipoproteins composed of PA and various proteins on the bitter taste of 0.5 mM quinine and 50 mM caffeine were examined (Table 1). PA-OVA had a suppressive effect similar to that of PA-LG. PA-BSA, PA-LA and PA-casein were less effective than PA-LG and PA-OVA, but significantly inhibited the bitterness of quinine. These lipoproteins also suppressed the bitterness of caffeine, but not as strongly as that of quinine. There was no essential difference in the effectiveness of the inhibition between the different lipoproteins. Therefore differences in the proteins of lipoproteins did not greatly affect the ability to inhibit the bitter taste.

Table 2 shows the effects of each component of PA-LG on the bitter taste of quinine and caffeine. LG itself had only a small suppressive effect on the bitterness of quinine and no effect on that of caffeine. The PA liposome greatly suppressed the bitterness of quinine, and hence PA itself can be used for the inhibition of the bitter taste of substances such as quinine. However, PA alone had no effect on the bitter taste of caffeine. A mixture of PA liposome and LG showed a large suppressive effect on the bitter taste of quinine similarly to PA-LG, but much less effect on the bitter taste of caffeine than PA-LG. That is, complex formation between PA and LG required to suppress the bitter taste of caffeine sufficiently. PA alone is not soluble in water, but PA-LG is easily dispersed in water and gives a transparent solution with only slight turbidity. Extraction of the PA-LG powder with *n*-hexane, which is a good solvent for PA, transferred only 5% of the PA to the solvent. This suggests that the PA in the powder is mostly bound to LG. Studies of the detailed physicochemical properties of PA-LG are in progress.

Figure 3 shows the effects of 3.0% PA-LG on the bitterness of various substances; R values varied among the bitter substances and ranged from 0.075 to 0.51. The bitter tastes of basic substances such as propranolol, promethazine, quinine and denatonium were greatly suppressed by PA-LG. The bitter tastes of brucine and strychnine, which are also basic, were less effectively suppressed than those of the other basic substances. The bitter taste of glycyl-*L*-leucine, caffeine, *L*-phenylalanine, naringin and theophylline was also suppressed by PA-LG. The effects of PA-LG were examined with two different concentrations of quinine, caffeine, naringin and theophylline. The bitter tastes of these substances at lower concentrations were more effectively suppressed than those at higher concentrations.

Table 1. Effects of various lipoproteins composed of PA and different proteins on bitter taste of 0.5 mM quinine and 50 mM caffeine. The values in the table represent R ± SE obtained from 8–10 volunteers. R was defined in Materials and Methods. Stimulating solutions were prepared by dissolving 0.5 mM quinine and 50 mM caffeine in 3.0% PA-LG solution.

Lipoprotein	Quinine	Caffeine
PA-LG	0.091 ± 0.013	0.428 ± 0.057
PA-BSA	0.204 ± 0.069	0.506 ± 0.067
PA-OVA	0.064 ± 0.011	0.415 ± 0.110
PA-LA	0.166 ± 0.043	0.466 ± 0.078
PA-casein	0.154 ± 0.031	0.452 ± 0.087

Table 2. Effects of each component of PA-LG on bitter taste of 0.5 mM quinine and 50 mM caffeine. The values in the table represent R ± SE obtained from 8–10 volunteers. R was defined in Materials and Methods. The concentration of PA-LG was 3.0%. The concentrations for PA liposome and LG were 0.85% and 2.15%, respectively, which were used to prepare 3.0% PA-LG. PA + LG is a simple mixture of PA and LG.

	Quinine	Caffeine
PA-LG	0.091 ± 0.013	0.428 ± 0.057
LG	0.792 ± 0.168	1.045 ± 0.107
PA liposome	0.142 ± 0.035	1.093 ± 0.153
PA + LG	0.095 ± 0.016	0.747 ± 0.184

In all the above experiments, the subjects tasted the bitter substances dissolved in solutions containing the lipoproteins. Hence the bitter substances may directly interact with the lipoproteins in the medium. To exclude this possibility, the tongue was first treated with PA-LG solution for 30 s or 2 min and then the subjects tasted the bitter substances dissolved in deionized water. As shown on Fig. 4, inhibition of the bitter taste of 0.5 mM quinine had already reached a steady level at 30 s and further inhibition was not brought about by a 2-min treatment. The extent of suppression brought about by this method was slightly less than that observed when quinine was tasted in the presence of PA-LG. The bitter taste of caffeine was suppressed to a greater extent by a 2-min than by a 30-s treatment. This extent of suppression by 2-min treatment was close to that observed when caffeine was tasted in the presence of PA-LG.

The data shown in Fig. 4 suggest that the suppression is brought about by masking of the target sites for bitter substances on the receptor membranes with PA-LG, but there is still a possibility that the suppression was partly brought about by direct interaction of the bitter substances with PA-LG in the medium when tasted in its presence. To test this possibility, binding of the bitter substances to PA-LG was measured. Bitter substances were dissolved in 3.0% PA-LG solution and the resultant solutions filtered with M. W. 5000 cut filters by centrifugation. The quantity of the bitter substance in the filtrate was determined by reverse phase chromatography. Table 3 shows the percentage of the quantity of a bitter substance in the filtrate to total quantity of the substance. The percentage was closely related to log P which represents the hydrophobic parameter of the molecule. That is, the binding of the substances to PA-LG was due mainly to hydrophobic interaction. Only less than 5% of propranolol, promethazine, quinine, brucine and strychnine remained in the filtrate, suggesting that these substances are mostly bound to PA-LG in the medium. These findings suggest that the suppression of the bitter taste of these substances is mostly due to the binding of the substances to PA-LG in the medium when dissolved in PA-LG solution for tasting. This is consistent with the finding that the bitter tastes of these substances were mostly suppressed by masking of their target sites with PA-LG when the tongue was treated with PA-LG and the bitter substances were tasted in the absence of PA-LG (see Fig. 4). Brucine and strychnine were strongly bound to PA-LG in the medium (Table 3), but the extent of the suppression was lower than that of the other hydropho-

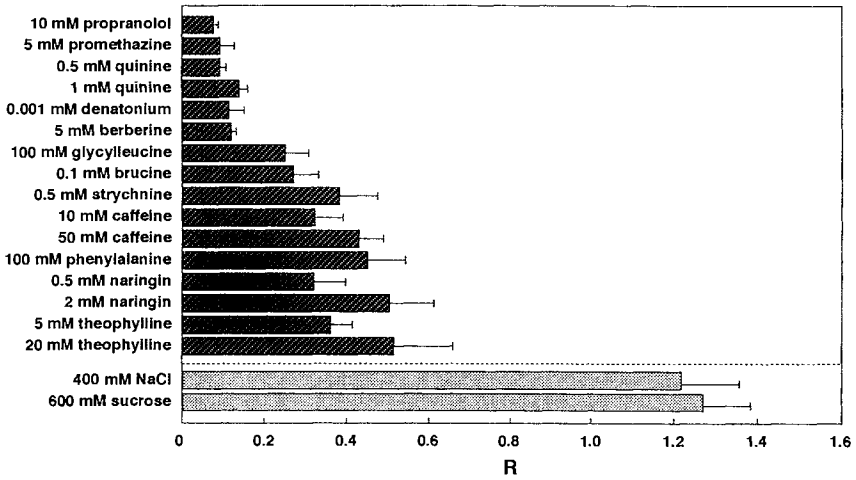

Figure 3. Effects of 3.0% of PA-LG on bitterness of various bitter substances, saltiness of NaCl and sweetness of sucrose. The relative taste sensation (R) was defined in Materials and Methods. Each column represents R ± SE obtained from 6-12 volunteers.

bic substances (Fig. 4). No bitter taste was observed at the moment of tasting of a solution containing PA-LG and brucine, but gradually a bitter taste was noted. This suggests that the complex between PA-LG and brucine is gradually dissociated into its components in the saliva.

In contrast to the hydrophobic substances, glycyl-*L*-leucine, caffeine, *L*-phenylalanine, naringin and theophylline are mostly unbound to PA-LG in the medium, which suggests that suppression of the bitter taste of these substances is brought about by masking of the target sites for the bitter substances with PA-LG. The percentage of the quantity of the unbound substance to the total quantity of the substance is not related to the extent of suppression of the bitter taste by PA-LG. For example, glycyl-*L*-leucine is not bound to PA-LG in the medium, but its bitterness was greatly suppressed by PA-LG (see Fig. 4). The bitter taste of propranolol was the most effectively suppressed by PA-LG among the bitter substances tested, but the affinity of propranolol to PA-LG is much lower than those of promethazine, quinine and brucine. This is because masking of the target sites for bitter substances with PA-LG contributes to the suppression of the bitter taste.

In previous studies, we found that PA-LG inhibits frog taste nerve responses to bitter substances without affecting the response to NaCl, galactose, acetic acid or *L*-alanine (9, 10). Here, the tongue was treated with a PA-LG solution containing no stimulus for 10 min and then a stimulus solution containing no PA-LG was applied to the tongue. Hence, inhibition of the responses to the bitter substances by PA-LG under these conditions is due to masking of the target sites for bitter substances with PA-LG. Treatment of the tongue with PA-LG for 30 s and subsequent stimulation of the tongue with bitter substance dissolved in PA-LG solution completely suppressed the responses to the bitter substances at much lower concentrations of PA-LG than that under the above experimental conditions. In most experiments in the

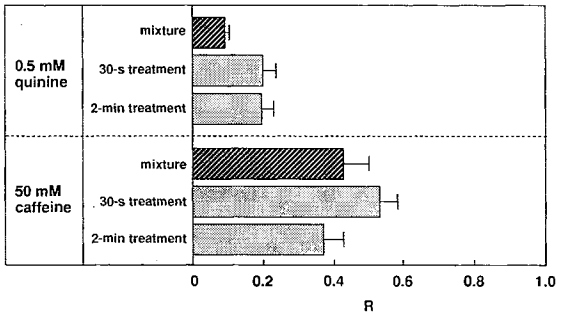

Figure 4. Effects of treatment of the tongue with 3.0% PA-LG on the bitter taste of 0.5 mM quinine and 50 mM caffeine. The tongue was treated with PA-LG solution for 30 s and 2 min and then the bitter substances dissolved in deionized water were tasted. "Mixture" represents data when the bitter substances dissolved in 3.0% PA-LG solution were tasted without treatment of the tongue with PA-LG, which were taken from Fig. 3. The relative taste sensation (R) was defined in Materials and Methods. Each column represents R ± SE obtained from 8-10 volunteers.

Table 3. Percentage of unbound bitter substance to PA-LG. Bitter substances were dissolved in 3.0% PA-LG solution, and the resultant solutions were filtered with M.W. 5000 cut filters by centrifugation. The quantity of the bitter substance in the filtrate was determined by reverse phase chromatography. The values in the table represent percentage of the quantity of the bitter substance in the filtrate to total quantity of the substance. LogP in the table represents hydrophobic parameter of the molecule, which was taken from the Pomona database. The parameters for denatonium and berberine were not found in the database.

Bitter substance	Unbound (%)	Log P
10 mM propranolol	4.60	2.57
5 mM promethazine	0.30	4.65
0.5 mM quinine	0.54	3.44
100 mM glyclyleucine	97.80	−1.96
0.1 mM brucine	0.60	0.98
0.5 mM strychnine	4.74	1.93
50 mM caffeine	100.00	−0.07
100 mM phenylalanine	97.10	−1.70
100 mM naringin	81.90	−2.13
20 mM theophylline	96.95	−0.02

present study, the tongue was not treated with PA-LG for practical reasons, but subjects tasted bitter substances dissolved in solutions containing PA-LG. Thus, the experimental conditions for studies on humans were different from those in frogs, but PA-LG was less effective on humans than on frogs.

Basic and hydrophobic substances such as quinine and denatonium have low taste thresholds and elicit a very bitter taste, and many drugs have such properties. PA-LG is most effective in suppressing the bitter taste of this type of substance. PA originates from soybean and LG, OVA, LA and casein originate from milk and eggs. Thus the lipoproteins can be safely used to mask the bitter taste of drugs. PA and these proteins are not connected by covalent bonds, but by hydrophobic interactions and hydrogen bonding. Hence, these lipoproteins should be easily hydrolyzed in the digestive system. PA-LG forms a complex with certain bitter substances, but the bitter substances seem to be easily released from PA-LG since LG is hydrolyzed in the digestive tract.

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