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Plasma protein binding of fentanyl: the effect of hyperlipoproteinaemia and chronic renal failure

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Hyperlipoproteinaemic patients with raised pre β-, or pre β- and β-lipoprotein fractions showed a significant ($P < 0.001$) increase in binding of fentanyl to whole plasma, compared with normal subjects. The presence of chylomicra had no significant effect on binding. In patients with chronic renal failure, a correlation of probability $P < 0.07$ was found between percent binding and concentrations of pre β-lipoprotein ($P = 0.001$), serum albumin ($P = 0.0101$), total protein minus albumin ($P = 0.0576$) and β-lipoprotein ($P = 0.0625$). There was no significant correlation of binding with elevation of α- or γ-globulins, with urea or creatinine concentrations, or with age or sex ($P \geq 0.223$). The magnitude of changes in the free fraction found in these patients should not produce a clinical effect as the total distribution volume of fentanyl exceeds 200 litres.

The short-acting opioid fentanyl has been shown to bind to albumin and lipoprotein fractions of plasma (Bower 1981). Pathological changes in the concentrations of these protein fractions may therefore cause altered binding. Fentanyl binding to plasma proteins was examined in conditions of hyperlipoproteinaemia and chronic renal failure to establish whether a change in the pharmacologically-active free fraction might promote an altered clinical response to the drug in such patients.

MATERIALS AND METHODS

The binding of fentanyl to whole plasma and to isolated lipoprotein fractions was measured at 37 °C, pH 7·4, by an equilibrium dialysis technique as previously described (Bower 1981).

Fentanyl

Tritiated fentanyl (8 Ci mM^{-1}) was obtained from I.R.E., Brussels, and prepared to a concentration of 0·6 ng ml^{-1} in 0·1 M sodium phosphate buffer. The concentration was chosen to represent a typical plasma concentration in the post-distributive phase after i.v. injection of 200 μg of the drug (Bower et al 1976).

Plasma

The binding of fentanyl to plasma of 15 normal volunteers (N1–15) was described by Bower (1981). Comparison is now made with results of 15 patients with chronic renal failure (R1–15), and of 6 patients with hyperlipoproteinaemia of Fredrickson type I, IIb, III, and IV (L1–6). The following clinical measurements were made on each sample of plasma: total protein, serum albumin, protein electrophoresis, fasting cholesterol and triglyceride and lipoprotein electrophoresis. Urea and creatinine concentrations were also measured in the patients with chronic renal failure.

The hyperlipoproteinaemic patients were treated by diet alone at the time of sampling. Patient L5 was tested at the time of diagnosis, and again after 3 months treatment with a low fat, low calorie diet. Renal patients taking few drugs were chosen, though binding of fentanyl is not affected by a wide range of drugs at clinical concentrations (Bower 1981). The renal patients were at the stage of preparation for regular haemodialysis which only patient R7 had begun, and he had not been dialysed for four days before plasma was taken.

To test the effect of chylomicra on binding, the results of a normal subject were compared before, and 5 h after, a fatty meal of fried food.

Isolation of lipoportein fractions

VLDL, LDL and HDL lipoprotein fractions were isolated from plasma by the flotation technique of Havel et al (1955), and prepared for equilibrium dialysis as previously described (Bower 1981).

Statistical analysis

Forward stepwise multiple regression analysis (MIDAS package, Statistical Research Lab., University of Michigan) was used to examine the correlation of binding with the clinical variables measured in the patients with chronic renal failure.

The number of hyperlipoproteinaemic patients found was insufficient for multiple regression analysis. Student's *t*-test was used for comparison of other results. Estimations of elevation of α- or β-globulins and lipoproteins, and of γ-globulins were assessed semi-quantitatively from electrophoretic strips, and scored for regression analysis as follows: no elevation score 0, + possible elevation 1, ++ definite elevation 2, +++ marked elevation 3.

RESULTS

The percent binding of fentanyl to whole plasma was significantly increased ($P < 0{\cdot}001$) in hyperlipoproteinaemic patients L2–6 compared with 15 normal subjects (Table 1). The results of patient L1, with only possible increase of pre β- and β-lipoprotein fractions, and of L5 after treatment, were not significantly different from normal. The values of total protein and serum albumin were within the normal range for all the hyperlipoproteinaemic patients.

The binding of fentanyl to the plasma of healthy volunteer N2 taken before, and 5 h after, a fatty meal, was not affected by the presence of chylomicra or the associated increase in triglyceride concentration from 1·2 to 2·7 (first occasion) and from 1·3 to 6·9 (second occasion). The serum cholesterol concentration was unaffected, and the lipoprotein concentrations were not raised by the test meal.

In the renal patients, correlation of clinical variables with percent binding by multiple regression analysis was significant at or below the 7% level for pre β-lipoprotein ($P = 0{\cdot}001$), serum albumin ($P = 0{\cdot}0101$), total protein minus albumin ($P = 0{\cdot}0576$) and β-lipoprotein ($P = 0{\cdot}0625$) (Table 2). Age, sex, and concentrations of urea, creatinine, α- and γ-globulins were not significantly correlated with percent binding ($P \geq 0{\cdot}223$). When the influence of the more highly correlated β- and pre β-lipoprotein concentrations was removed by multiple regression analysis, the residual contributions of cholesterol and triglyceride concentrations were not significant.

Binding to the recovered VLDL and LDL fractions was significantly increased in pooled plasma from hyperlipoproteinaemic patients L2 and L4, and from renal patients R5 R10 and R11 with raised lipoproteins ($P < 0{\cdot}001$) (Table 3). Binding to the recovered HDL was not significantly different from normal in the renal patient pool, but was significantly reduced ($P < 0{\cdot}01$) in the hyperlipoproteinaemic patients. The mass of HDL recovered was similar in all three groups.

DISCUSSION

The importance of lipoprotein fractions in the binding of fentanyl to plasma proteins has been established (Bower 1981), and is reflected and confirmed by the increased binding shown by patients with hyperlipoproteinaemia (Table 1).

The concentrations of total protein and serum albumin of these patients were normal, and the fatty meal experiment in a normal subject showed that the presence of chylomicra does not affect binding of fentanyl. After 3 months treatment by diet, the pre β- and β-lipoprotein concentrations of patient L5 returned to normal, and the binding of fentanyl fell to a normal value (Table 1). Therefore the elevated

Table 1. Percent binding (mean ± s.e.m.) of fentanyl to plasma, and clinical values in six hyperlipoproteinaemic patients compared with results of 15 normal subjects.

No.	Sex	Age	Type	Cholesterol mM	Triglyceride mM	Chylomicra	Electrophoresis	Lipoprotein electrophoresis	B%
L_1	M	35	IV	7·8	1·8	—	β++	preβ+ β+	79·12 ± 0·37†
L_2	M	34	IV	10·8	19·0	—	α_2+ β++γ++	preβ+++ β+	84·40 ± 0·40*
L_3	M	30	I	12·0	32·7	+++	α_2+	preβ+++ β‡	85·33 ± 0·26*
L_4	M	60	IIb	8·0	4·5	++	α_2+	preβ++ β+	82·40 ± 0·19*
L_5	F	45	IIb	9·7	3·6	—	Normal	preβ++ β++	83·60 ± 0·32*
L_6	F	49	III	11·4	8·4	++	β+	broad β band	83·73 ± 0·29*
L_5 (treated)	F	45	IIb	6·5	1·6	—	Normal	Normal	78·88 ± 0·41†
Normal subjects N 1–15 (Bower 1981)				5·89 ± 0·21 s.e.m.	1·17 ± 0·08 s.e.m.	—	Normal	Normal	79·16 ± 0·16

* The difference is significant at the 0·1% level with respect to the result for normal subjects.
† The difference is not significant at the 5% level with respect to the result for normal subjects.
‡ Specimen grossly lipaemic, β-band obscured.

Table 2. Percent binding (mean ± s.e.m.) of fentanyl to plasma proteins, and clinical values in 15 uraemic patients

No.	Sex	Age	Total protein g/l	Albumin g/l	*'Rest' g/l	Urea mM	Creatinine mM	Cholesterol mM	Triglyceride mM	Electrophoresis	Lipoprotein electrophoresis	B%
R_1	M	41	72	40	32	68·2	1504	5·9	3·5	α_2++	pre β++	81·50 ± 0·12
R_2	F	33	67	40	27	38·6	716	7·9	3·3	α_2++	pre β++ β+	81·57 ± 0·43
R_3	M	33	59	36	23	31·4	618	6·0	1·2	normal	normal	73·73 ± 0·37
R_4	M	38	66	41	25	34·2	886	5·2	3·1	α_2+	pre β++	81·80 ± 0·40
R_5	F	57	61	21	40	37·8	802	9·7	2·7	α_2++ γ++	pre β+ β++	78·00 ± 0·31
R_6	M	34	76	45	31	10·9	1144	9·8	3·0	γ+	pre β++ β++	84·67 ± 0·35
R_7	M	20	61	33	28	15·2	1054	5·7	3·2	α_2++ γ++	pre β++ α^-	80·03 ± 0·29
R_8	F	54	69	42	27	28·5	848	7·5	3·2	normal	pre β++ β+	81·87 ± 0·19
R_9	M	21	52	29	24	31·5	1360	5·2	2·5	α_2++ γ+	pre β+	72·67 ± 0·88
R_{10}	M	51	65	30	35	23·5	920	8·7	2·4	α_2+	pre β+ β++	78·66 ± 0·32
R_{11}	M	51	57	31	22	25·1	800	10·0	2·3	α_2++ γ++	pre β+ β++	77·70 ± 0·57
R_{12}	M	20	59	33	26	34·0	1260	6·2	1·1	α_2++ γ++	normal	71·93 ± 0·33
R_{13}	M	43	53	29	24	43·0	560	6·1	2·0	α_2++	pre β+	73·20 ± 0·42
R_{14}	F	53	67	41	26	29·2	520	6·3	2·6	α_2+	pre β+	75·38 ± 0·53
R_{15}	M	47	57	34	23	33·0	910	6·2	1·8	α_2+ γ++	pre β+	71·38 ± 0·34

* 'Rest' = Total protein minus albumin

Correlation coefficient of B% with pre β = 0·82219 (P = 0·001), with albumin = 0·70731 (P = 0·0101), with total protein minus albumin = 0·5612 (P = 0·0576) and with β = 0·55247 (P = 0·0625).

VLDL and LDL fractions found in these patients appear to be responsible for the enhanced binding of fentanyl.

Only in patient L1, with a possible increase of pre β- and β-lipoproteins, was binding not significantly different from normal (Table 1). A more marked increase in these fractions seems to be necessary in order to affect the binding of fentanyl. This is supported by the fact that in healthy subjects N1–15, binding was not correlated to normal variation in fasting concentrations of cholesterol and triglyceride, which reflect the β- and pre β-lipoprotein fractions respectively (Bower 1981).

There was no significant difference between patients L2, with a predominant rise in the pre β-fraction, and L5 and L6 with elevation of pre β- and β-bands. The effect seems to be related to total lipoprotein elevation.

Table 3. The percent binding of fentanyl (mean ± s.e.m.) to lipoproteins (very low density VLD, low density LD, high density HD) in normal volunteers, renal and hyperlipoproteinaemic patients.

Subject pool	B% VLDL	LDL	HDL
N2 3 5 7 8	18·39 ± 0·65	37·14 ± 0·42	21·18 ± 0·51
R5 10 11	26·82 ± 0·43*	54·63 ± 0·87*	22·75 ± 0·38†
L2 4	42·31 ± 0·47*	43·72 ± 0·33*	17·45 ± 0·28*
Mg% lipoprotein recovered			
N2 3 5 7 8	124·1	398·6	416·3
R5 10 11	162·3	468·4	352·1
L2 4	208·7	415·8	379·6
Normal values (range)			
M	159 ± 116	369 ± 81	275 ± 79
F	56 ± 60	303 ± 36	436 ± 140
From Hatch & Lees (1968)			

* The difference is significant at the 0·1% level with respect to the results of normal subjects.
† The difference is not significant at the 5% level with respect to the results of normal subjects.

The patients had an average free fraction of 16%, compared with 21% for normal subjects (Table 1). The pharmacologically active free fraction is therefore reduced by 20%.

Danon & Chen (1979) have shown increased binding of imipramine to plasma of hyperlipoproteinaemic patients, with increased binding to the raised lipoprotein fractions. A correlation of total percent bound and concentrations of serum cholesterol ($P > 0·0003$) and triglyceride ($P < 0·001$) was reported.

The effect of chronic renal failure on the plasma protein binding of drugs is dependent upon the chemistry of the drug. Binding of many acidic drugs is reduced, e.g. sulphonamides (Anton & Corey 1971), phenytoin (Odar-Cederlof et al 1970), and salicylates, phenylbutazone and thiopentone (Andreasen 1973). However, many basic drugs such as quinidine, dapsone and desmethylimipramine show normal binding to plasma of uraemic patients, even where the same plasma has been shown to give reduced binding of the acidic phenytoin or fluorescein (Reidenberg & Affrime 1973).

The factors which may affect drug binding in renal failure include hypoalbuminaemia, abnormal albumin configuration, hyperlipoproteinaemia, and displacement by accumulated endogenous and exogenous compounds including drug metabolites. Reidenberg et al (1971) have shown a significant correlation ($P < 0·05$) between binding of phenytoin to plasma proteins of uraemic patients and concentrations of serum albumin and total serum protein. However the magnitude of the decrease of phenytoin binding was greater than that predicted by simple dilution of plasma with phosphate buffer (Lunde et al 1970).

Indeed the binding of sulphonamides in uraemia may be low when the concentrations of serum proteins are normal (Scholtan 1961; Boobis 1977). Shoeman et al (1973) have given evidence of altered albumin configuration in uraemia by isoelectric focussing. Boobis (1977) also reported the presence of an abnormal albumin band on isoelectric focussing, and described qualitative changes in amino acid content. These authors found reduced association constants between phenytoin (Shoeman et al 1973) and sulphadiazine (Boobis 1977) and uraemic albumin.

In contrast, normal or slightly increased binding has been reported in uraemic patients for many basic drugs. Binding of quinidine to plasma of uraemic rats was shown to be associated with increased binding to albumin, expressed as a function of albumin concentration, and occurred despite hypoalbuminaemia (Nilsen et al 1975). The association constant and number of binding sites were not determined.

Binding of fentanyl to plasma of uraemic patients included values within, above and below the normal range, and was correlated with concentrations of serum albumin ($P < 0{\cdot}0101$), pre β-lipoprotein ($P < 0{\cdot}001$) and β-lipoprotein ($P < 0{\cdot}0625$) (Table 2). Since many basic drugs bind to lipoproteins, the hyperlipoproteinaemia often associated with chronic renal failure may help to offset any factors, including hypoalbuminaemia, that tend to decrease binding. Increased binding of fentanyl to raised concentrations of VLDL and LDL occurred (Table 3). Nilsen et al (1975) also reported increased binding of quinidine to raised VLDL in uraemic rats. Patients with hypoalbuminaemia but little or no elevation of lipoproteins showed the lowest values of binding of fentanyl (patients R3, R9, R12 and R15), while highest binding was found in patients with raised lipoproteins, and serum albumin concentrations within the normal range of 34–50 g litre^{-1} (R1, R2, R4, R6 and R8) (table 2). Acidic drugs do not bind to lipoproteins, and therefore hyperlipoproteinaemia in uraemic patients will not tend to offset factors which cause reduced binding.

Accumulated inhibitors of drug binding, which may include metabolites, have been demonstrated in uraemia by Andreasen (1974). The increase in binding of phenytoin, phenylbutazone, and sulphadiazine after dialysis in vitro was significantly greater in plasma from uraemic patients than in normal donors.

There is evidence of non-dialysable endogenous inhibitors for salicylic acid and warfarin (Sjöholm et al 1976), and chloramphenicol (Vodrazka et al 1978). These authors reported restoration of normal binding to uraemic plasma after charcoal treatment at pH 3. This suggests a role for a tightly bound anion such as free fatty acid (FFA). Concentrations of FFA are often raised in renal failure due to increased mobilization of adipose tissue (Losowsky & Kenward 1968). Competition by raised FFA for binding sites on albumin may inhibit drug binding, or reduced binding may be due to the allosteric effect of FFA on albumin (Soltys & Hsia 1977).

In the case of fentanyl, binding to plasma proteins is not affected by the observed concentrations of urea and creatinine, raised FFA, changes in concentration of the common ions of plasma, the presence of the metabolites of fentanyl, or clinical concentrations of other drugs (Bower 1981). The correlation of binding with concentrations of urea and creatinine, and therefore with the severity of renal failure, was not significant in the 15 uraemic patients. There was no significant correlation of binding with age, sex, α- or γ-globulin elevation.

Differences of fentanyl binding are therefore found in uraemic and hyperlipoproteinaemic patients which, though slight, are significantly different from normal, and reflect changes in the plasma protein fractions in these patients. Such changes are proportionally greater when expressed in terms of percent change in the pharmacologically active free fraction. However the free drug will equilibrate throughout the total apparent volume of distribution of the drug, which for fentanyl is in excess of 200 litres (Bower et al 1976). The final change of free drug concentration at the receptor sites is therefore negligible, and no change in clinical response would be expected in these patients due to the observed changes in binding. Other aspects of these conditions, particularly chronic renal failure may, however, affect the pharmacodynamics of fentanyl by other mechanisms.

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