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Radioimmunoassay of the new opiate analgesics alfentanil and sufentanil. Preliminary pharmacokinetic profile in man

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The development of two analogous radioimmunoassay (RIA) procedures based on dextran-charcoal separation is described for the quantification of two fentanyl-like analgesics, alfentanil and sufentanil. Immunization of rabbits with conjugates of bovine serum albumin and carboxy-derivatives of the respective drugs resulted in the production of antisera capable of detecting less than 0·05 ng ml^{-1} of the parent analgesics with high specificity and almost no cross-reactivity with major metabolites. Excellent agreement was obtained between RIA—without prior extraction—and gas chromatography for alfentanil concentrations in human plasma. Because of sufentanil's low therapeutic plasma levels, no comparison could be made between its RIA and an alternative assay, however, there was strong evidence for the specificity of the assay when applied directly to plasma. With these RIA methods preliminary information was obtained on plasma concentrations and elimination of alfentanil or sufentanil in patients given an intravenous bolus injection of 50 μg kg^{-1} of alfentanil, or 5 μg kg^{-1} of sufentanil. For both analgesics, the pharmacokinetic profile in man could be described by a three-compartment model. The terminal elimination half-life was 88 min for alfentanil and 140 min for sufentanil. Six hours after a therapeutic dose, plasma levels were in the order of 3 and 0·3 ng ml^{-1} for alfentanil and sufentanil respectively.

Alfentanil, *N*-[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4- piperidinyl]-*N*-phenylpropanamide monohydrochloride, is a potent and very short acting intravenous narcotic analgesic, chemically related to fentanyl. In the tail withdrawal test of analgesia in rats alfentanil was about 5 times less potent than fentanyl. The duration of action was about 10 min and the onset of action was more rapid than that of fentanyl (Niemegeers & Janssen 1981).

Sufentanil, *N*-[4-(methoxymethyl)-1[2-(2-thienyl)-ethyl]-4-piperidinyl]-*N*-phenylpropanamide 2-hydroxy-1,2,3-propanetricarboxylate (1:1) is a potent narcotic analgesic with an analgesic potency about ten times that of fentanyl and an exceptional safety margin (Niemegeers et al 1976). Analgesic doses administered to surgical patients were in a range of 0·5 to 5 μg kg^{-1} (Bovill et al 1981).

For the study of the pharmacokinetics of both analgesic compounds after either bolus injection or continuous infusion (alfentanil), a sensitive and reliable assay capable of determining the expected low plasma concentrations was required.

We describe the development and the evaluation of radioimmunoassay procedures, for alfentanil and sufentanil. The value of the assays for pharmacokinetic studies was demonstrated by their application in assessing the disposition of the analgesics in man. The alfentanil assay was further evaluated by comparing the plasma concentrations obtained with those measured by a specific gas chromatographic procedure.

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MATERIALS AND METHODS

Specifically tritium-labelled alfentanil (spec. act. 16·1 Ci mM^{-1}) and sufentanil (spec. act. 15·0 Ci mM^{-1}) were synthesized by selective dehalogenation of the appropriate precursors at I.R.E. (Fleurus, Belgium) according to procedures developed in our laboratory. The radiochemical purity was checked with radio-h.p.l.c. (Waters Associates with a Berthold LB 504 radioactivity detector) and was found to be ≥99% for either drug. Both compounds were stored as ethanolic solutions at −20 °C.

Dextran-radioimmunoassay grade was purchased from Schwarz-Mann Labs, Orangeburg, N.Y.; bovine albumin (Cohn fraction V) from Sigma, St Louis, Mo; Norit A-supra from Norit N.V., Amersfoort, The Netherlands; 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride from Janssen Chimica, Beerse, Belgium and Pico-fluor 30 from Packard, Belgium.

All drugs and test compounds were originally

synthesized and analysed in the Janssen Research Laboratories, Beerse, Belgium.

Preparation of the hapten-derivatives

To a stirred and refluxing mixture of 15 parts of 1-ethyl-1,4-dihydro-4-[2-[2-(methoxymethyl)-4-(phenylamino)-1-piperidinyl]ethyl]-5*H*-tetrazol-5-one, 1 part of *NN*-dimethyl-4-pyridinamine and 640 parts of 4-methyl-2-pentanone, was added dropwise 9·5 parts of methyl 4-chloro-4-oxobutanoate with stirring continued for 2 h at reflux temperature. The residue from the cooled and evaporated reaction mixture was suspended in water and made alkaline with 1 M sodium hydroxide at < 5 °C. The product was extracted twice with dichloromethane, the combined extracts washed with water, filtered and evaporated. A mixture of 3·24 parts of the reaction product and 50 parts of 1 M sodium hydroxide was stirred for 3 h in an oil bath at 80 °C. The reaction mixture was cooled and acidified with acetic acid to pH 5·5 at < 5 °C.

The aqueous phase was decanted and the residual oil was crystallized from a mixture of 4-methyl-2-pentanone and 2,2'-oxybispropanone. After filtration, the product was recrystallized from 2-propanone, yielding 1·5 parts of R 50 115 or 4-[[1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-4-piperidinyl]-phenylamino]-4-oxobutanoic acid (m.p.: 156·9 °C; elemental analysis calculated for $C_{22}H_{32}N_6O_5$; N, 18·25; C, 57·38; H, 7·00; found, N, 17·73; C, 57·21; H, 7·05).

Similarly, a hapten derivative of sufentanil was synthesized starting from 4-(methoxymethyl)-*N*-phenyl-1-[2-(2-thienyl)ethyl]-4-piperidinamine, yielding R 50 127 or 4-[[4-(methoxymethyl)-1-[2-(2-thienyl)ethyl]-4-piperidinyl]phenylamino]-4-oxobutanoic acid (m.p. 156·1 °C; elemental analysis, calculated for $C_{23}H_{30}N_2O_4S$; N, 6·51; C, 64·16; H, 7·02; S, 7·45; found: N, 6·31; C, 63·76; H, 7·03; S, 7·48).

Preparation of the immunogens

An amount of 100 mg of the respective hapten derivatives was dissolved in 2 ml of dimethylacetamide and added dropwise to 400 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, dissolved in 5 ml of distilled water. This mixture was added to 200 mg of bovine serum albumin (BSA) dissolved in 30 ml of 0·1 M phosphate buffer, pH 6·0. The resulting solution was stirred overnight at 4 °C. The reaction mixture was dialysed extensively against 0·05 M phosphate buffer of pH 7·4 for about 16 h at 4 °C and then freeze-dried. A schematic representation of the procedures is outlined in Fig. 1.

Immunization

The resulting protein conjugates were dissolved in phosphate buffered saline (0·05 M), pH 7·4, at 1 mg ml^{-1} and emulsified on a Vortex mixer with an equal volume of complete Freund's adjuvant. Female New Zealand white rabbits, two per conjugate, were immunized with 1·5 ml of the emulsion by multiple intradermal injections along each side of the back (Vaitukaitis et al 1971). Beginning one month after the priming dose, booster injections were given at 6-week intervals. Eight days after the 4th booster, large blood samples of up to 60 ml were collected from each animal from the orbital venous plexus.

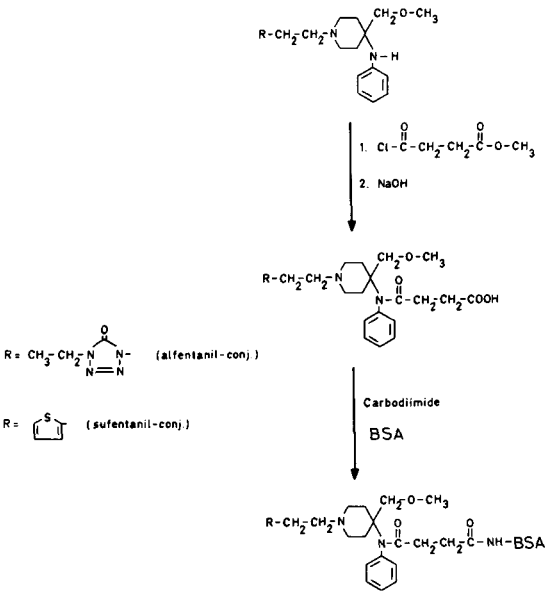


FIG. 1. Synthesis of the hapten derivatives and the alfentanil- and sufentanil-protein conjugates used for immunization of rabbits.

This procedure was repeated after each booster injection until sufficient serum was collected. The rabbits were killed 16 months after the first immunization, 8 days after the last booster. The rabbit serum was titrated for antibodies against the respective drugs, pooled per compound and stored at −20 °C.

Assay of alfentanil and sufentanil

The procedure for immunoassay of alfentanil or sufentanil was essentially the same as that described for pimozide or fentanyl (Michiels et al 1975, 1977). Antiserum titres were determined by adding 0·2 ml

of serially diluted rabbit serum to alfentanil to 0·5 ml phosphate buffer (0·05 M, pH 7·4) and 0·5 ng of [^{3}H]alfentanil (27 000 d min^{-1}), contained in 0·05 ml of 30% methanol–water. Incubations were carried out in 1·3 ml plastic tubes (Eppendorf) by continuous rotation (25 rev min^{-1}) for 2 h at room temperature (20 °C). Thereafter, bound and free [^{3}H]alfentanil were separated by selective adsorption on dextran-coated charcoal: aliquots of 0·2 ml of a suspension containing 2 g activated charcoal (Norit), 200 mg dextran and 100 mg sodium azide per 100 ml BSA-phosphate buffer (0·05 M, pH 7·4) were added to the incubation mixture and allowed to equilibrate at room temperature for 1 h with continuous rotation. The charcoal was removed by centrifugation at 8 000 *g* for 10 min (Microfuge, Heraeus-Christ). The supernatant, containing antibody-bound [^{3}H]alfentanil, was pipetted into a minivial containing 3 ml of Pico-fluor 30 and the radioactivity determined in a liquid scintillation spectrometer (Packard-Prias). By external standard ratios and a counting efficiency curve, counts min^{-1} were converted by programmed analysis to d min^{-1} (Wang PCS 2200 II 4-2, Wang Labs. Inc., Mass.). Appropriate controls were included to determine non-specific binding, for which undiluted normal rabbit serum was substituted for the antiserum.

Similarly, serum from rabbits injected with the sufentanil conjugate were tested for antibodies to sufentanil. [^{3}H]Sufentanil (0·2 ng, corresponding with 17 000 d min^{-1}) was used as the radioligand.

Standard curves of alfentanil or sufentanil were obtained by incubating increasing amounts of unlabelled drug together with fixed amount of the radioligand (0·5 ng[^{3}H]alfentanil or 0·2 ng [^{3}H]sufentanil) in the presence of 0·2 ml of a dilution of the respective antisera which bound nearly 30–35% of the tracer, as found by previous titration. Incubations were carried out in BSA-buffer by measuring the inhibition of the antibody-alfentanil or antibody-sufentanil complex formation produced by increasing amounts of up to 1000 ng of the identified and postulated metabolites of either compound (Meuldermans et al 1982) and some structurally related compounds.

The inter- and intra-assay variability and the accuracy of the procedure were tested over 3 weeks by repetitive analysis of alfentanil and sufentanil standards, added to control human plasma and by assaying increasing volumes of a same plasma sample. All samples were assayed in duplicate and results were calculated as outlined by Rodbard (1974) using a Wang desk computer system for iterative weighed linear regression analysis of logit B/Bo versus log dose.

Procedure in man

Alfentanil was injected intravenously as a bolus in patients at 50 and 125 μg kg^{-1}. Venous blood samples were collected in heparin before, and from 1 min to 6 h after, bolus injection. Plasma was separated by centrifugation at 2500 rev min^{-1} for 10 min and stored at −20 °C until analysis. Plasma alfentanil concentrations were determined by radioimmunoassay, either directly or after selective extraction, using antibodies directed to alfentanil. Drug concentrations were calculated from simultaneously run standard curves processed in the same way as the unknown samples. The extraction procedure for alfentanil standard added to control plasma or for unknown samples was as follows: 0·5 ml aliquots of plasma were made alkaline (pH 11–12) with 2 ml of 0·01 M sodium hydroxide and extracted twice with 2 ml of heptane–isoamyl alcohol (95:5). The combined organic layers were evaporated to dryness. The residues were dissolved in 0·5 ml of blank (outdated) human plasma. After vigorous mixing on a Vortex, appropriate aliquots of these solutions were taken for the assay of alfentanil. The over-all yield of the procedure was 92·3% as determined with the labelled drug and was concentration-independent.

Comparison with gas chromatography. Plasma samples of patients treated at 50 or 125 μg kg^{-1} were analysed by radioimmunoassay without prior extraction; and by gas chromatography of plasma extracts according to Woestenborghs et al (1981).

Sufentanil was given intravenously as a bolus injection to patients at 0·5 and 5 μg kg^{-1}. Venous blood samples were collected in heparin before and from 1 min up to 6 h after injection. Plasma was centrifuged and stored at −20 °C until analysis. Plasma sufentanil concentrations were determined by radioimmunoassay, either directly on plasma or after selective extraction, using antibodies raised specifically to sufentanil. The extraction procedure was similar to that for alfentanil. The over-all yield was 86·4% as determined with the labelled drug and was independent of the concentration. Drug concentrations were calculated from standard curves run simultaneously and processed in the same way as the unknown samples.

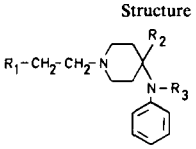
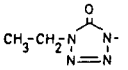
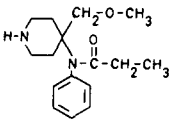
RESULTS

Radioimmunoassay: alfentanil

Assay characteristics. The sera of both rabbits injected with the alfentanil-immunogen contained antibodies capable of binding [^{3}H]alfentanil as demonstrated by antiserum titrations. Nearly 35% of the added tracer bound specifically to antibodies at 1/550 dilution of the pooled alfentanil antisera. Non-specific binding to control rabbit serum was less than 1·5% at every dilution. Following logit transformation a standard curve for unlabelled alfentanil added to control plasma gave a linear response from about 0.05 to 5 ng if the limit of significance was restrained to 10% inhibition of [^{3}H]alfentanil binding.

The specificity of the antiserum towards various structural congeners and possible metabolites of alfentanil is shown in Table 1. The cross-reaction (ID50) is expressed as the molar ratio of the amount of a compound required to inhibit [^{3}H]alfentanil binding to antibodies by 50% relative to the amount of radioligand. The antibody did not discriminate between alfentanil and the hapten-derivative. Other modifications at the site of attachment of the hapten to the protein carrier drastically diminished the ability of these analogues to displace [^{3}H]alfentanil from antibody sites as illustrated by some possible metabolites formed by oxidative attack at the methoxymethyl moiety. The metabolite noralfentanil, which by analogy with fentanyl was formed by oxidative *N*-dealkylation at the piperidine (Meuldermans et al 1982), did not interfere with radioligand binding. Desproprionylalfentanil, a synthetic precursor of alfentanil and possible degradation product formed by amide hydrolysis of the parent drug, showed cross-reaction only at 150-fold molar excess. Fentanyl, sufentanil, carfentanil and lofentanil did not bind to any measurable degree to alfentanil antibodies (Table 2).

Table 1. Antibody specificity: competition between various possible metabolites and [^{3}H]alfentanil or [^{3}H]sufentanil for binding to the respective antibodies. Cross-reaction is expressed as the molar ratio of alfentanil or sufentanil and the test compound required to inhibit by 50% complex formation between the tracer and the antiserum (ID50).

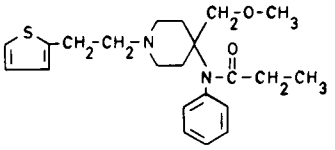
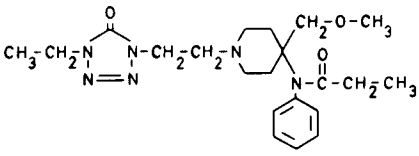
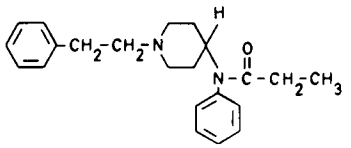
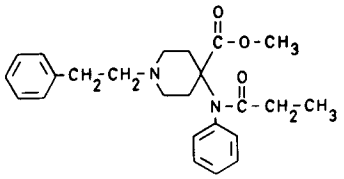
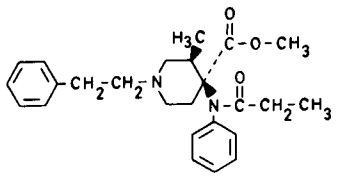
Structure		R_1	
		alfentanil 	sufentanil 
R_2	R_3	ID50	
$-CH_2-O-CH_3$	$-C(=O)-CH_2-CH_3$	1·0	1·0
$-CH_2-O-CH_3$	$-C(=O)-CH_2-CH_2-COOH$	1·2 hapten	1·1 hapten
$-CH_2-O-CH_3$	$-H$	50	43
$-CH_2-OH$	$-C(=O)-CH_2-CH_3$	12	29
$-CH_2-OH$	$-H$	—	73
$-COOH$	$-C(=O)-CH_2-CH_3$	—	417
$-H$	$-C(=O)-CH_2-CH_3$	288	61
		>1000	>1000

The intra- and inter-assay coefficients of variation (c.v.) were 3·7 and 3·3% respectively in a range of 0·09 to 2·2 ng per test tube. To insure that the radioimmunoassay gave a quantitative measure of alfentanil, known amounts added to control human plasma were measured at various occasions. An excellent correlation was found between calculated and measured alfentanil concentrations (slope = 0·941; r = 0·999) and c.v.-values were less than 9% (22 determinations of each pool). The linear regression for the correlation of the sample volume versus the estimated amount demonstrated that the estimates were independent of both concentration and sample dilution with respect to interference which may be present in the sample (r = 0·999; range 0·05–0·5 ml; concentration 2·5 ng ml^{-1}).

Assay validation. The validity of the radioimmunoassay for alfentanil in human plasma samples was demonstrated by the assay, either directly or after selective extraction of the samples, pooled per interval, from patients injected intravenously at 50 μg kg^{-1}. The regression line calculated for the plasma concentration of alfentanil determined after extraction versus alfentanil levels measured directly in pooled plasma samples, showed a slope of 1·27 and an intercept of nearly 1·0. The correlation coefficient was 0·998 over a concentration range of 2 to 540 ng ml^{-1} (n = 12). Correction for extraction recovery was achieved by the preparation of standard curves of alfentanil standard solutions added to control human plasma and processed in the same way as the unknown samples. Further validation was obtained by comparative analysis by radioimmunoassay applied directly to, and by gas chromatographic analysis of, plasma samples from surgical patients dosed with the drug at 50 or 125 μg kg^{-1}.

An excellent correlation was found (r = 0·998)

Table 2. Antibody specificity: competition between chemically related analgesics and [3H]alfentanil or [3H]sufentanil for binding to antibodies directed to alfentanil or sufentanil. Cross-reaction is expressed as the molar ratio of alfentanil or sufentanil and the test compound required to inhibit by 50% complex formation between the tracer and the antiserum (ID50).

Reference No. (generic name)	Structure	ID50 alfentanil	ID50 sufentanil
R33800 sufentanil		>1000	1
R39209 alfentanil		1	>1000
R4263 fentanyl		>1000	135
R33799 carfentanil		>1000	3·7
R34995 lofentanil		>1000	418

between the results obtained by radioimmunoassay without prior extraction and by the specific gas chromatographic method ($\log y = 0{\cdot}96 \log x + 0{\cdot}07$, range 5–1670 ng ml^{-1}, n = 22). These results obviously demonstrate the absence of metabolic interference with the direct radioimmunoassay of human plasma samples.

Radioimmunoassay: sufentanil

Assay characteristics. Both rabbits injected with the sufentanil-BSA conjugate developed antibodies capable of binding [3H]sufentanil. Nearly 31% of the added tracer (0·2 g) bound specifically to antibodies at 1/4000 dilution of the pooled antisera, whereas non-specific binding was less than 1·5%. When applied to plasma, as little as 25 pg of sufentanil could be detected and assayed accurately. Under the present conditions the assay was linear up to 5 ng ml^{-1}. The specificity of the antiserum to sufentanil is shown in Table 1. The procedure followed to determine cross-reaction was similar to that of the alfentanil antiserum.

As was expected the antibodies did not discriminate between [3H]sufentanil and the hapten-derivative. Some postulated metabolites which could be formed by oxidation and ultimate decarboxylation at the methoxy-methyl-moiety interfered with [3H]sufentanil binding from 29 to 417-fold molar excess. Despropionylsufentanil, a synthetic precursor and possible degradation product which might be formed by amide hydrolysis of the parent drug, and also the desmethyl-analogue displaced 50% of the radioligand at 43 and 73-fold excess respectively. The metabolite norsufentanil, which by analogy with fentanyl was formed by oxidative *N*-dealkylation, did not interfere with antibody-binding of [3H]sufentanil.

Whereas fentanyl antibodies failed to bind sufentanil at measurable concentrations (Michiels et al 1977), the antiserum to sufentanil cross-reacted with fentanyl at a ratio of 1 to 135 (Table 2). Lofentanil (R 34 995) showed an ID50-value of 418 whereas alfentanil did not bind to any measurable degree to sufentanil antibodies. On the other hand, carfentanil, differing from sufentanil by substitution of phenyl for thiophene and a carboxymethyl moiety for the methoxymethyl, bound to antibody sites to almost the same extent as did [3H]sufentanil (ID50 = 3) (Table 2).

Intra- and inter-assay coefficients of variation (c.v.) were 4·7 and 3·9% respectively over a range of 0·07 to 1·6 ng per assay tube. There was a good correlation between calculated and measured sufentanil concentrations in a series of controls added to human plasma (r = 0·999, slope = 0·998) and c.v.-values were less than 9% (22 determinations of each pool). Serial dilutions of human plasma samples of

high sufentanil concentration demonstrated that the estimates were independent of the concentration and sample dilution (r =0·999, range 0·05–0·5 ml, concentration 2·4 ng ml^{-1}).

Assay validation. Plasma samples from surgical patients after intravenous injection of sufentanil at 0.5 or 5 μg kg^{-1} were assayed either directly or after liquid-liquid extraction at pH 11. The slope of the regression line calculated for plasma concentrations of sufentanil determined after extraction versus sufentanil levels measured directly in plasma was 0·85 and the intercept was 0·173. The correlation coefficient was 0·992 over a range of 0·1 to 20 ng ml^{-1} (n = 47). A slight discrepancy between direct and indirect assay was observed in the sense that with the direct assay, throughout the investigational period (0–8 h) a systematic over-estimation of about 20% was found with respect to plasma levels measured after extraction.

Experiments in man

The mean plasma concentrations of alfentanil in two subjects after an intravenous bolus at 50 μg kg^{-1} are shown in Fig. 2. Plasma alfentanil concentrations decreased rapidly from 540 ng ml^{-1} at 1 min to about 38 ng ml^{-1} at 1 h after drug administration. From 1 h the drug was eliminated from plasma with a mean half-life of about 88 min. Concentrations of alfentanil in plasma could still be easily measured with the radioimmunoassay for up to 6 h after injection.

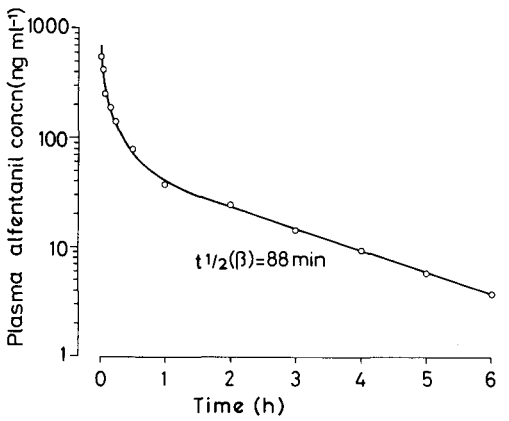


FIG. 2. Mean plasma alfentanil concentrations in surgical patients (n = 2) after intravenous administration of alfentanil at 50 μg kg^{-1}. Concentrations were determined by RIA applied directly to plasma samples.

As for alfentanil, the time-course of the plasma levels of sufentanil in man after intravenous bolus injection of 5 μg kg^{-1} declined triexponentially and could be described by a three-compartment model with rapid and extensive distribution to tissues (Bovill et al 1981). From 1 h, sufentanil was eliminated from plasma with a half-life of 140 min. Six hours after injection, plasma sufentanil concentrations were still 0·26 ng ml^{-1} (Fig. 3).

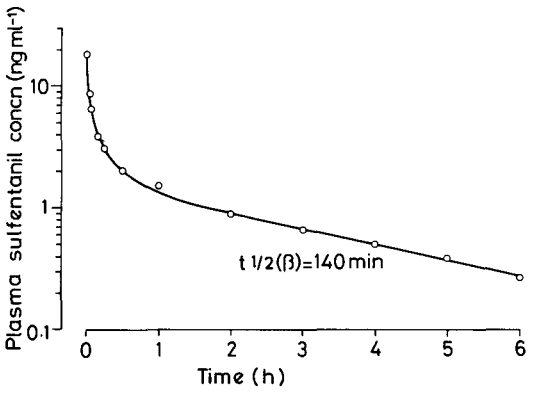


FIG. 3. Mean plasma sufentanil concentrations in surgical patients (n = 2) after intravenous administration of sufentanil at 5 μg kg^{-1}. Concentrations were determined by RIA applied directly to plasma samples.

DISCUSSION

The aim of this paper has been to evaluate the radioimmunoassay procedures and to prove their applicability in clinical and pharmacokinetic studies. The limit of detection of the assays was 50 pg for alfentanil and 25 pg for sufentanil which makes the assays suitable for the measurement of plasma concentrations of the drugs, especially after low therapeutic doses. The gas chromatographic procedure is substantially less sensitive (Woestenborghs et al 1981) since it was unable to measure reliably either drug in plasma below 1–2 ng ml^{-1}, although it was suitable for measuring alfentanil concentrations after higher doses.

All postulated metabolites of either compound with the intact alkylpiperidinyl nucleus have been traced by comparative radio-h.p.l.c. analysis in urine and faeces from rats and dogs and none of them have been found in detectable amounts. Major identified metabolic pathways were the oxidative *N*-dealkylation to the common nor-analogue and the oxidative *O*-demethylation at the methoxymethyl

moiety (Meuldermans et al 1982). Therefore, the hapten derivatives for alfentanil and sufentanil were designed with only minor modification of the propionyl chain, analogous to that of fentanyl (Henderson et al 1975; Michiels et al 1977). The specificity of the respective antibodies was shown by their ability to differentiate the parent drugs from other closely related compounds including metabolites (Table 1). In view of this, drugs with a chemical structure differing from that of the fentanyl-like analgesics and which may be co-medicated during anaesthesia, should not interfere in the assay of either alfentanil or sufentanil.

The good agreement between plasma concentrations in humans obtained by direct assay or after extraction demonstrates the specificity of either antiserum and suggests that extraction of the parent compound from plasma is not required. This was confirmed for alfentanil by the perfect correlation between plasma concentrations in man measured by both gas chromatography and RIA applied directly to the plasma samples. For sufentanil a systematic over-estimation of about 20% was observed between determinations on sample extracts or on unextracted plasma. Since this discrepancy occurred from the earliest sample intervals and persisted throughout the investigational period, this might point to incomplete recovery rather than to possible interference of metabolites.

The antibody directed to alfentanil failed to bind to any extent sufentanil, fentanyl, lofentanil or carfentanil. The antibody to sufentanil only slightly bound fentanyl and lofentanil, but failed to bind alfentanil. Carfentanil, however, was hardly discriminated from [^{3}H]sufentanil (Table 2). The data on sufentanil and carfentanil (Table 2) show that substitution of phenyl for thiophene did not dramatically alter binding to sufentanil antibodies whereas replacement by tetrazole (alfentanil) completely abolished antibody binding. The latter may be explained also by very large differences in the physicochemical properties. The introduction of optically active sites, as for lofentanil, the methyl-analogue of carfentanil, represented a drastic change of the antigenic determinant. Omission of the methoxymethyl moiety of sufentanil resulted in a decreased antibody binding (ID50 = 61,Table 1). For the phenylethyl-analogue of this dealkylated compound, i.e. fentanyl, the amount required to inhibit [^{3}H]sufentanil binding increased an additonal two-fold (Table 2). So, in spite of the very similar tridimensional configuration of the atoms in sufentanil and fentanyl (Fig. 4), the large differences in charge distribution may strongly influence antibody binding. Also, differences in ionization may be reflected in different antibody binding. For sufentanil, it can be calculated that in physiological medium (pH 7·4) about 20% of the drug present is non-ionized whereas for fentanyl only 8·5% is present as the free base (Tollenaere et al 1979).

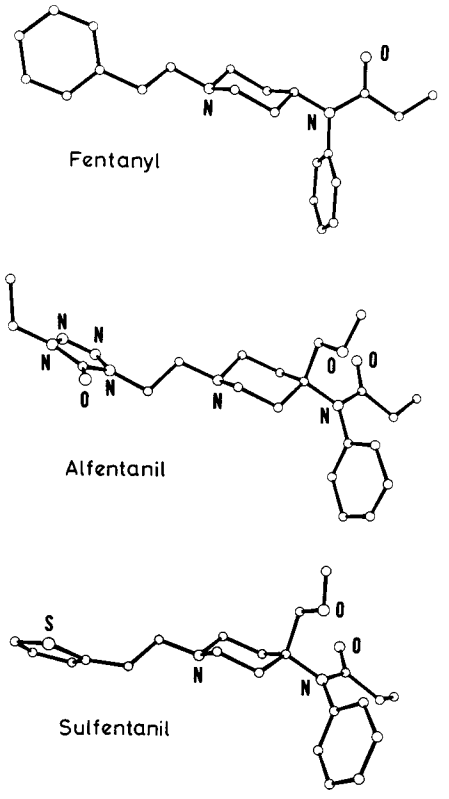


FIG. 4. Tridimensional representation of the fentanyl, alfentanil and sufentanil molecules (Tollenaere et al 1979).

The RIA procedures described have been successfully applied to the study of sufentanil (Bovill et al 1981) and alfentanil pharmacokinetics in man (Bovill et al 1981; Schüttler & Stoeckel 1982; Bower & Hull 1982; Camu et al 1982) and to the development of infusion models for alfentanil in man (Schüttler et al 1982).

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