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IMMUNOASSAY DETECTION OF DRUGS IN RACING HORSES. XI. ELISA AND RIA DETECTION OF FENTANYL, ALFENTANIL, SUFENTANIL AND CARFENTANIL IN EQUINE BLOOD AND URINE

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SUMMARY

We have developed and evaluated a one step enzyme-linked immunosorbent assay (ELISA) test for sufentanil and a ^{125}I radioimmunoassay test for alfentanil as part of a panel of pre- and post-race tests for narcotic analgesics in racing horses. Our sufentanil ELISA test detects sufentanil with an I-50 of about 0.5 ng/ml. The test is rapid and economical in that it can be read with an inexpensive spectrophotometer, or even by eye. The test readily detects the presence of sufentanil or its metabolites in equine blood and urine from 1 to 24 hours respectively after administration of therapeutic or sub-therapeutic doses of this drug. Our sufentanil assay also cross-reacts with fentanyl, the methylated analogs of fentanyl (designer fentanyls), and carfentanil and detected these drugs in urine for several hours after their administration to horses. It does not, however, cross-react significantly with alfentanil.

We have also developed an ^{125}I radioimmunoassay for alfentanil. This test allows detection of alfentanil in blood and urine of horses for up to 4 hours after administration of this drug. As such, these tests are capable of improving the quality and reducing the cost of pre-race and post-race testing for fentanyl, sufentanil, carfentanil and alfentanil and a number of their congeners in racing horses. Similarly, these tests are capable of screening for these drugs in human drug abuse monitoring.

INTRODUCTION

Fentanyl (N-phenyl-N-[1-(2-phenylethyl)-4-piperidinyl]propanamide) is a synthetic opioid derivative of meperidine and is a potent narcotic analgesic, with about 80 to 150 times the potency of morphine (Tobin et al., 1979; Jaffe and Martin, 1985). Its narcotic actions are characterized by rapid onset and short duration of action. The pharmacologic actions are similar to those of morphine, and fentanyl is considered a pure opioid agonist (Martin, 1984).

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Fentanyl has been widely used in human medicine as a short acting narcotic analgesic, particularly in association with surgical procedures (Holderness et al., 1975). More recently the manufacturers of fentanyl have developed several analogs of fentanyl for different medical uses. These analogs include alfentanil and sufentanil which are used in human medicine, and carfentanil, a highly potent analog of fentanyl for use in animal capture and zoo animal medicine. Lofentanil, another fentanyl analog, has not been released for any medical or commercial use (Janssen, 1985). Other analogs of fentanyl, the so called designer drugs, ∝-methylfentanyl and 3-methylfentanyl, are illegally synthesized for the illicit market and also present abuse problems in both human and veterinary science.

Sufentanil and alfentanil are now routinely used medically (Greeley et al., 1987; Shafer et al., 1986). As such, they are relatively readily available, and they have the potential to be illicitly diverted into human and equine drug abuse. Similarly, carfentanil is available to certain veterinary professionals, and as such may be diverted to illicit use in both the equine and human areas. Lofentanil, an analog of fentanyl with no commercial outlets or approved uses, is less likely to be diverted into illicit markets. For all of these drugs, however, there is a significant probability of diversion of these agents, and we have therefore developed simple and rapid screening tests for these drugs in equine samples.

We have based our approach to these screening tests on models previously developed by our group (Woods et al., 1986; Tai et al., 1988; Tobin et al., 1988b). We have developed ^{125}I radioimmunoassay (RIA) tests for alfentanil and a one step enzyme-linked immunosorbent assay (ELISA) test for sufentanil. Additionally, these antibodies can be readily incorporated into the particle concentration fluoroimmunoassay (PCFIA) format if this is

required. Based on our previous experience in horse racing, these assays should allow rapid and effective screening for these agents in both equine and human urine samples.

MATERIALS AND METHODS

Biological Samples

Equine blood and urine samples were obtained from mature Thoroughbred, half Thoroughbred and Standardbred horses (400-600 kg) kept at pasture and allowed free access to food and water. The horses were placed in standard box stalls (17 m^2) approximately 12 hours prior to dosing for acclimatization.

Post-race urine samples from racing horses were collected by the authorities in charge at the individual racecourses and delivered to the drug testing laboratory of the racing jurisdiction.

Dosing and Sampling

Authentic fentanyl citrate, carfentanil citrate, alfentanil hydrochloride, sufentanil citrate, ∝-methylfentanyl, and 3-methylfentanyl drug standards were provided by Janssen Life Sciences (Piscataway, NJ). Fentanyl was injected into the horse as fentanyl citrate (Sublimaze Injection^R, Janssen), sufentanil as sufentanil citrate (Sufenta Injection^R, Janssen), and carfentanil as carfentanil citrate (Wildnil Injection^R, Wildlife Laboratories, Fort Collins, CO). Alfentanil hydrochloride, -methylfentanyl and 3-methylfentanyl were dissolved in sterile saline for injection. All injections were by rapid IV injection into the jugular vein.

All urine samples were collected by bladder catheterization. Blood samples were collected by venipuncture into vacuum blood tubes containing potassium oxalate and sodium fluoride (for plasma) or serum separation vacuum tubes (Becton Dickinson Vacutainer Systems, Rutherford, NJ). The

blood samples were centrifuged to collect the plasma and serum. All samples were stored frozen until assayed.

Radioimmunoassay

RIA for alfentanil was carried out as described by Tai et al. (1988). Briefly, this assay is based on an antibody to alfentanil and the iodinated (^{125}I) analogs of this drug. The sample volume was 50 µl/tube. Alfentanil derivatives were supplied courtesy of Dr. David Watt of the University of Kentucky Department of Chemistry.

One Step ELISA Tests

The one step ELISA tests were performed as previously described (Voller et al., 1976; Yang et al., 1987; McDonald et al., 1988). Briefly, anti-fentanyl antibody was linked to flat bottom Immulon Removawells^{R} (Dynatech, Chantilly, VA) as described by Voller. Similarly, sufentanil was linked to horseradish peroxidase (HRP) (Pradelles et al., 1985), to give rise to a covalently linked sufentanil-HRP complex. All reactions were run at room temperature.

The assay was started by adding 20 µl of the standard, test or control (phosphate buffer, pH 7.4) samples and 180 µl of the reagents to microtiter wells on a microplate. During this step the presence of free drug or cross-reacting metabolites competitively prevented the anti-sufentanil antibody from binding to the sufentanil-HRP conjugate. The degree of antibody-fentanyl HRP binding was therefore inversely proportional to the amount of drug in the sample. After incubation, the fluid was removed from the microtiter wells and the wells washed three times with buffer. TMB Microwell Peroxidase substrate solutions (Kirkegaard and Perry, Gaithersburg, MD) were then added to all wells and their absorbance read at 650 nm in a microplate reader (Bio-Tek Instruments, Winooski, VT), when the optical density of a well containing no added drug reached about 1.0.

Carfentanil Extraction

To 1 ml of sample (serum or urine) in a microcentrifuge tube was added 3 drops of $NaHCO_3$/NaOH solution (0.1N $NaHCO_3$, NaOH added to pH 12-13). The tube was capped and well mixed. Chloroform/isopropanol (3:1) (400 ul) was added, followed by mixing. The tubes were centrifuged at 8800 x g for 30 sec in an Eppendorf Microcentrifuge (Brinkman Instruments, Westbury, NY). The top layer was removed and discarded. The bottom layer was transferred into a glass culture tube and evaporated in a 40 °C water bath under a stream of N_2. Methanol (25 µl) and assay buffer (150 µl) were added to the tube with mixing after each addition.

RESULTS

The data of Figure 1 show the time course and sensitivity to added sufentanil of our ELISA test. In the absence of added sufentanil the reaction rapidly runs to completion, with an apparent absorbance value of about 1.6 being attained between 50 and 60 minutes after starting the

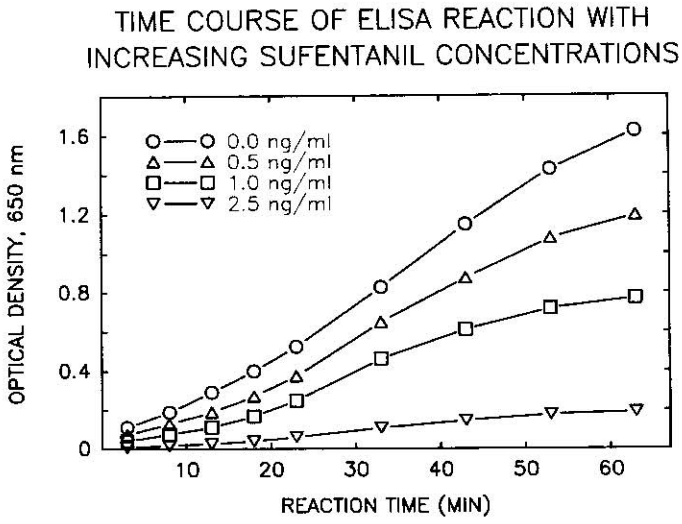

Figure 1. The ELISA reaction was inhibited by the addition of increasing concentrations of sufentanil citrate. The sample was 20 µl of phosphate buffer, pH 7.4.

reaction. The addition of increasing concentrations of sufentanil acted to inhibit the reaction, with virtually complete inhibition of the reaction occurring after addition of 2.5 ng/ml of sufentanil.

Because these assays are usually run on serum or urine samples added directly to the sample wells, we studied the effect of addition of equine serum and urine samples to our ELISA system. A small inhibition of the reaction rate was seen after addition of equine urine (Figure 2), and there was little or no inhibition after addition of human urine or equine or human serum.

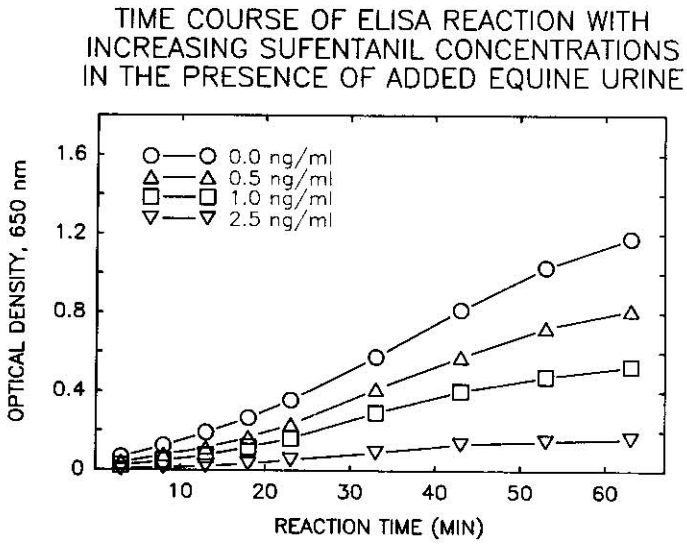

Figure 2. The addition of 20 µl of blank equine urine slowed the ELISA reaction, but the added urine did not reduce the inhibition by the increasing concentrations of sufentanil.

Figure 3 shows the cross-reactivity of our anti-sufentanil antibody in the ELISA format. Sufentanil half-maximally inhibits the reaction at

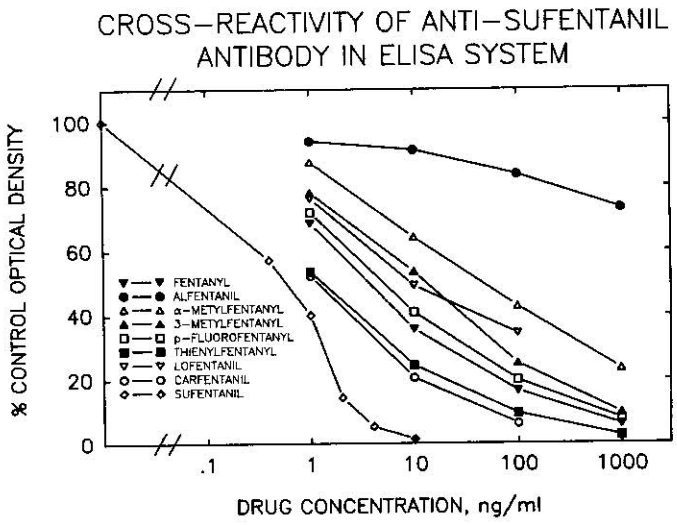

Figure 3. The ELISA system based on the anti-sufentanil antibody was tested for sensitivity to a series of fetanyl analogs. "Control" optical density was the reading when no drug was added to the system.

concentrations of about 0.5 ng/ml, and the anti-sufentanil antiserum cross-reacts well with carfentanil and thienylfentanyl (I-50 = 1 ng/ml). p-Fluorofentanyl and fentanyl itself are the next strongest cross-reacters, with half-maximal inhibition occurring at about 5 ng/ml. The methylfentanyls and lofentanil are less cross-reactive, with concentrations of more than 10 ng/ml required for half-maximal inhibition. Based on the convenience of the ELISA, this format of the sufentanil appears to offer advantages in screening for the fentanyls.

This ELISA test is suffiently sensitive to detect sufentanil in equine plasma after administration of small doses of this drug to horses (Figure 4). The ELISA test was almost completely inhibited at five minutes after

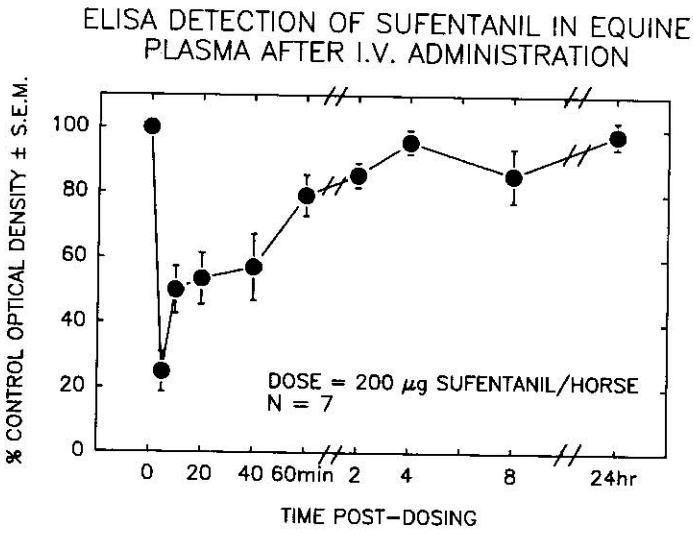

Figure 4. The plasma sufentanil equivalents of a horse dose with 200 μg sufentanil IV as measured by ELISA. Each sample was assayed 7 times and the interassay standard error of the mean (SEM) was calculated. "Control" optical density was the reading when no drug was added.

drug administration but inhibition declined rapidly thereafter. By sixty minutes after drug administration, inhibition of the ELISA was no longer apparent, consistent with the expected pharmacokinetic pattern of a highly lipid soluble drug.

Urinary detection of sufentanil (Figure 5) followed a broadly similar pattern. For the first two hours after dosing with sufentanil citrate virtually complete inhibition of the ELISA test was observed, with the inhibition declining thereafter at a rate dependent on the dose. When a 0.05 mg dose was administered, the urinary inhibition had returned to control by between 8 and 24 hours after administration. If the dose of sufentanil was larger, the urine contained significant quantities of sufentanil equivalents, producing about 40% inhibition of the ELISA test at 24 hours after dosing.

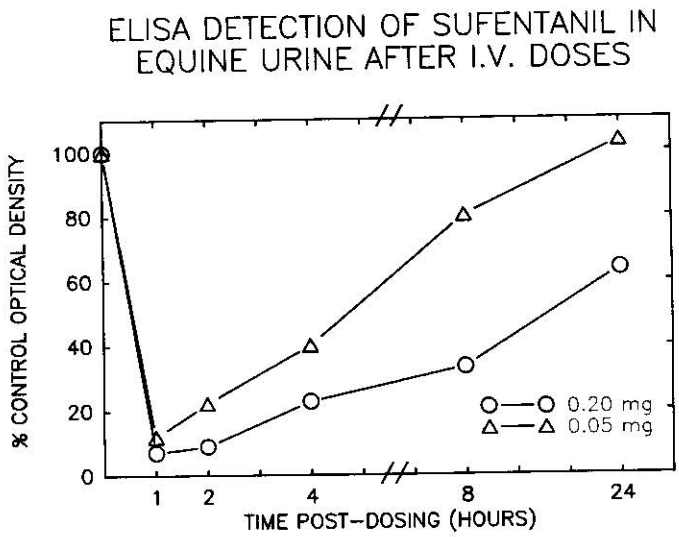

Figure 5. The urine sufentanil equivalents concentrations of 2 horses dosed IV with 200 μg (O-O) and 50 μg/horse (Δ - Δ) sufentantil was determined by ELISA. "Control" optical density was the reading when no drug was added.

The sufentanil ELISA was able to detect sufentanil equivalents in equine urine after intravenous administration of fentanyl or its methylfentanyl analogs (Figure 6). Inhibition of the ELISA test was observed for between 8 and 24 hours after a dose of 0.5 mg fentanyl, 3-methylfentanyl or α-methylfentanyl per horse. Therefore, this ELISA should be useful in detection of abuse of these members of the fentanyl family of drugs.

A series of 48 horse urine samples, included blank urine samples and urine samples from horses dosed with carfentanil and sufentanil were assayed for sufentanil equivalents by ELISA (Table 1). A "positive" was assigned if the sample yielded an ELISA value less than the mean minus 2 standard deviations of the readings of the blank samples. Our ELISA test detected all three fentanyl samples and all but three of the carfentanil samples. The three "false negative" carfentanil samples were early time points at

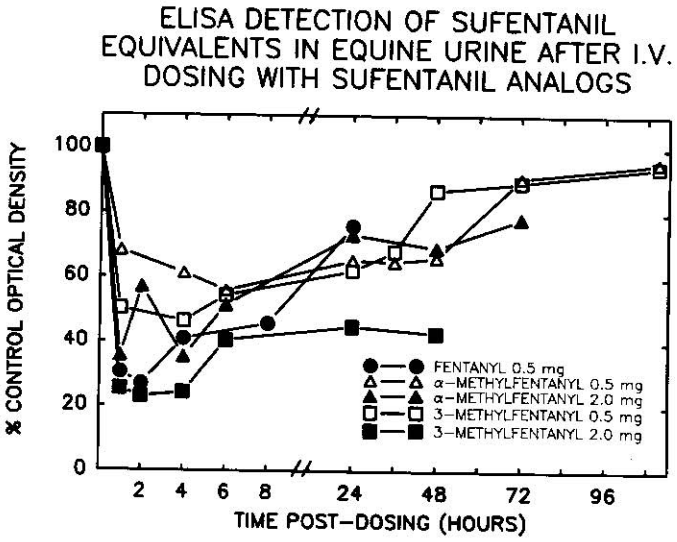

Figure 6. The urine sufentanil equivalents concentration from horses dosed with fentanyl, α-methylfentanyl and 3-methylfentanyl were determined by ELISA. "Control" optical density was the reading when no drug was added.

lower doses of the drug, where the concentrations of the carfentanil metabolites in the urine were likely to be low.

Because of the importance of carfentanil as a potential illegal medication, we evaluated the ability of our sufentanil ELISA to detect carfentanil in blood and urine after its administration to horses. If the samples were subjected to extraction prior to assay, carfentanil could be detected in equine serum for up to one hour after administration of the 100 µg/horse dose (Figure 7). Similarly, if urine samples were extracted, both 50 and 100 µg/horse doses were readily detected in equine urine for between one and four hours after drug administration (Figure 8). With the addition of a simple extraction step, therefore, this sufentanil assay should detect administration of fentanyl, its methylated analogs, and also sufentanil and carfentanil.

Table 1. Detection of fentanyl and carfentanil by sufentanil ELISA.

Forty-eight urine samples were assayed by the sufentanil ELISA method. The second column indicates the samples that were from horses dosed with fentanyl or carfentanil, the dosage level and the time post-dose of the sampling. In this column, "-" represents a blank urine sample. The ELISA optical density reading is listed in the third column. The ELISA status of each dosed horse sample was determined by comparison to the mean value minus 2 times the standard error of the mean of the blank urines (0.714). "-" indicates a negative status; "**" indicates a false positive; "***" indicates a false negative.

Sample #	Treatment	Optical Density	Status
1	-	0.814	-
2	-	0.779	-
3	Carfentanil, 160 µg, 2-4 h	0.577	Positive
4	-	1.149	-
5	Fentanyl, 100 µg, 2-4 h	0.401	Positive
6	-	0.800	-
7	-	0.945	-
8	-	0.746	-
9	-	0.798	-
10	-	0.913	-
11	-	0.635	Positive**
12	-	1.055	-
13	Carfentanil, 100 µg, 0-1 h	0.757	-***
14	-	0.915	-
15	-	1.188	-
16	-	0.982	-
17	-	1.048	-
18	Fentanyl, 100 µg, 0-2 h	0.192	Positive
19	-	1.051	-
20	Carfentanil, 160 µg, 1-2 h	0.758	-
21	-	0.792	-
22	-	1.095	-
23	-	0.884	-
24	-	1.200	-
25	-	0.807	-
26	Carfentanil, 125 µg, 1-2 h	0.824	-***
27	-	1.063	-
28	-	1.000	-
29	-	0.977	-
30	Carfentanil, 160 µg, 0-1 h	0.672	Positive
31	Carfentanil, 125 µg, 0-1 h	0.826	-***
32	Fentanyl, 50 µg, 1-2 h	0.381	Positive
33	-	1.191	-
34	-	1.071	-
35	-	1.013	-
36	Carfentanil, 100 µg, 2-4 h	0.555	Positive
37	-	1.006	-
38	-	0.900	-
39	-	0.982	-
40	Carfentanil, 160 µg, 4-6 h	0.631	Positive
41	-	1.056	-
42	Carfentanil, 100 µg, 4-6 h	0.585	Positive
43	-	1.086	-
44	-	1.121	-
45	Carfentanil, 125 µg, 2-4 h	0.555	Positive
46	-	1.119	-
47	-	1.003	-
48	-	1.032	-

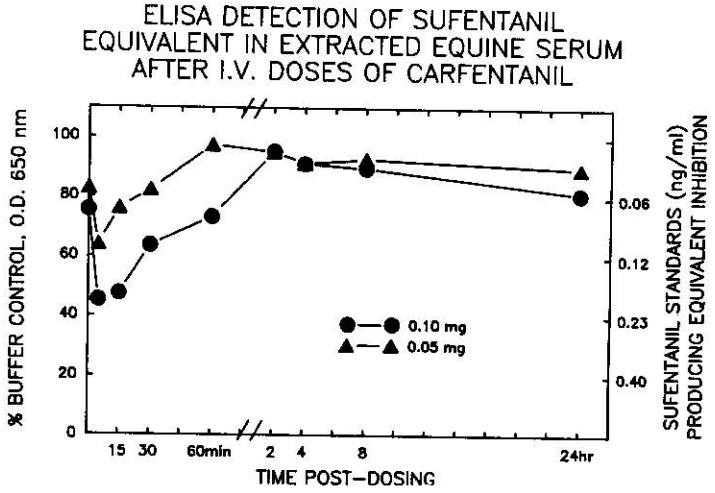

Figure 7. The serum sufentanil equivalents concentrations of 2 horses dosed IV with 100 µg (●-●) and 50 µg (▲-▲) carfentanil, following extraction, determined by ELISA. "Buffer control" optical density was the reading when no drug was added.

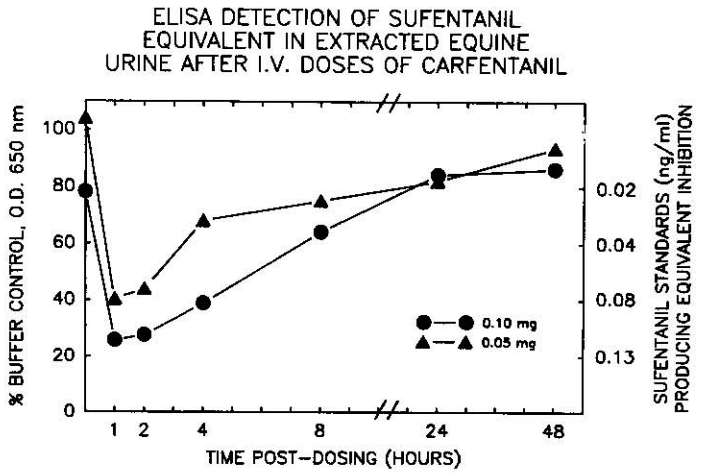

Figure 8. The urine sufentanil equivalents concentrations of 2 horses dosed IV with 100 µg (●-●) and 50 µg/horse (▲-▲) carfentanil, following extraction, was determined by ELISA. "Buffer control" optical density was the reading when no drug was added.

While our sufentanil antibody cross-reacts well with most of the fentanyl analogs, it shows virtually no cross-reactivity with alfentanil. We therefore raised an antibody to alfentanil and constructed an RIA for alfentanil using our anti-alfentanil antiserum and ^{125}I-alfentanil (Figure 9). This RIA is able to detect alfentanil down to about 0.2 ng/ml with an apparent I-50 of 2.2 ng/ml. The alfentanil antibody in the RIA system was also found to cross-react with two alfentanil derivatives (Figures 10 and 11). The antibody was more sensitive to Derivative B (I-50 = 6.5 ng/ml) than Derivative A (I-50 = 55 ng/ml).

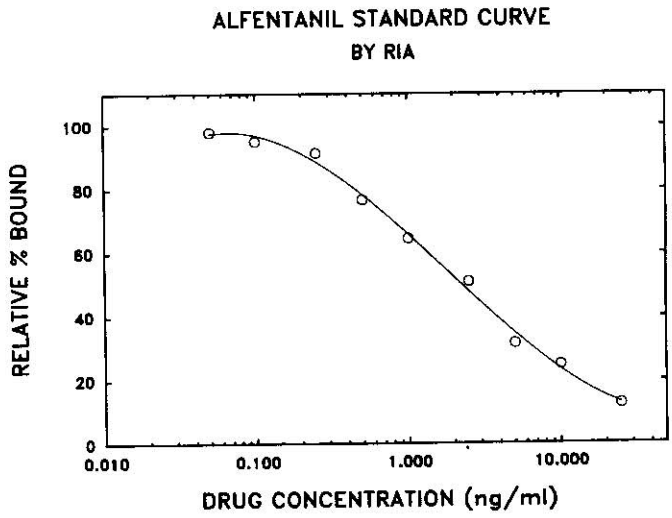

Figure 9. Radioimmunoassay standard curve for the ^{125}I-alfentanil assay. The I-50 for alfentanil in this system was about 2.2 ng/ml.

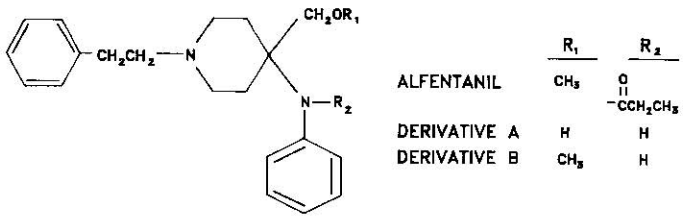

Figure 10. Structures of alfentanil and of alfentanil derivatives A and B.

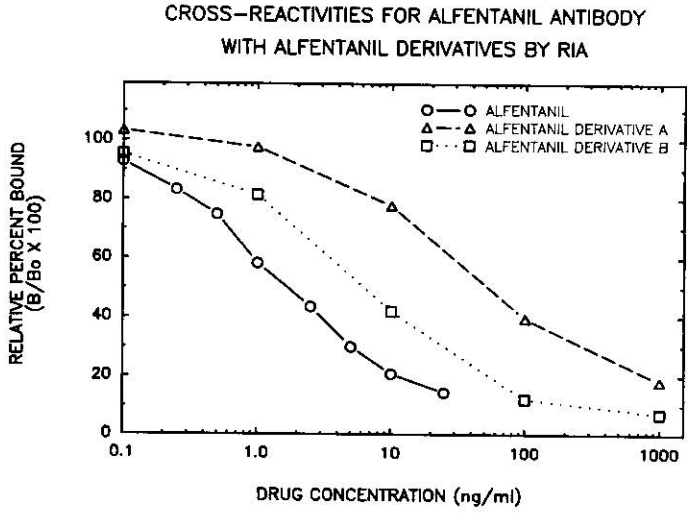

Figure 11. The cross-reactivity of the anti-alfentanil antibody in the RIA system was tested for alfentanil (0-0), alfentanil derivative A (Δ-Δ), and alfentanil derivative B (□-□). The I-50's for derivatives A and B were about 55 and 6.5 ng/ml respectively.

The alfentanil antibody was tested for cross-reactivity in the RIA system with several fentanyl analogs (Figure 12). There was no significant cross-reactivity with fentanyl, ∝-methylfentanyl, 3-methylfentanyl, sufentanil or carfentanil.

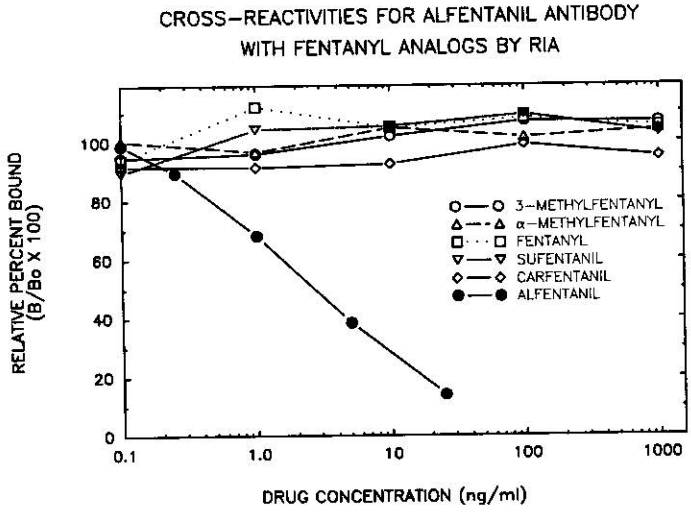

Figure 12. The cross-reactivity of the anti-alfentanil antibody in the RIA system was determined for a series of fentanyl analogs.

Two horses were dose intravenously with 0.5 mg alfentanil hydrochloride per horse. The alfentanil RIA was used to detect alfentanil equivalents in the blood and urine of these horses. The assay was able to detect alfentanil in plasma (Figure 13) for about 1 hour post-dose with mean peak level of about 0.5 ng/ml at 30 min. By 2 hours post-dose the alfentanil equivalents concentration in plasma had returned to the pre-dose values.

Alfentanil equivalents were detected in the urine of horses dosed with 0.5 mg alfentanil for up to 4 hours post-dose (Figure 14). The peak concentration was at 1 hr for horse #1 at about 0.13 ng/ml and at 30 min for horse #2 at about 0.35 ng/ml.

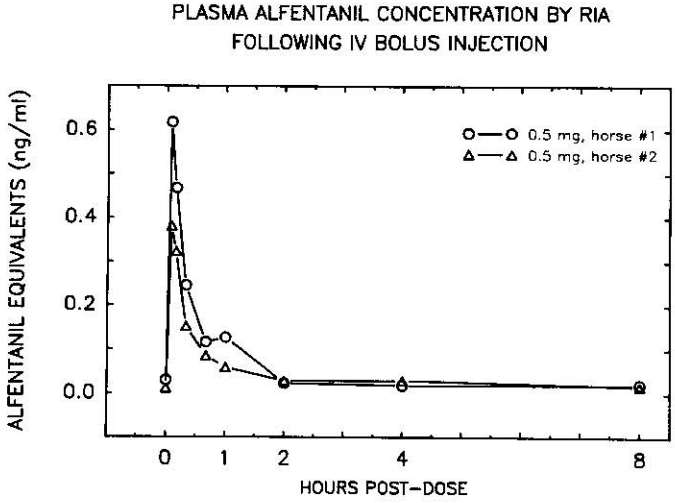

Figure 13. The plasma alfentanil equivalents concentration of 2 horses dosed IV with 0.5 mg alfentanil was determined by ^{125}I-alfentanil RIA.

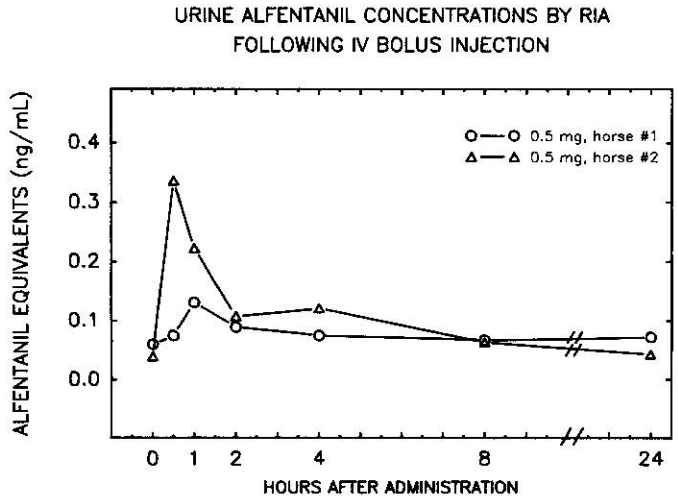

Figure 14. The urine alfentanil equivalents concentration of 2 horses dosed IV with 0.5 mg alfentail was determined by ^{125}I-alfentanil RIA.

DISCUSSION

We have raised an antibody to sufentanil that detects sufentanil in the low nanogram-high picogram/ml range and also reacts well with sufentanil metabolites in equine urine. Additionally, this antibody cross-reacts well with fentanyl itself, and at least two of the methylated analogs of fentanyl. It also reacts sufficiently well with carfentanil to allow detection of administration of this drug in equine blood and urine if a simple extraction/concentration step is used in the development of this assay. Because the sensitivity and cross-reactivity of the only other commercially available immunoassay for sufentanil are not well characterized, we elected to develop this antibody into an ELISA and an RIA. Because of the superior performance of ^{125}I-RIA tests in quantitative work, the RIA we are currently developing will likely be more useful as a research tool. On the other hand the broad spectrum of drugs detected and the ease of the use of the ELISA test makes this format a particularly useful screening assay.

As a screening test with the capability of detecting the presence of sufentanil, fentanyl, the methylated fentanyls and carfentanil, this assay should be particularly useful in racing chemistry. As a group, the fentanyls are readily available because of their widespread use in human medicine. For this reason, their diversion into horse racing is not uncommon, and during the late 1970's fentanyl was widely abused in horse racing. With the development of an RIA for fentanyl (Woods et al., 1986) and the concommitant development of a gas chromatography/mass spectrometry (GC/MS) confirmation method for fentanyl, abuse of this drug was brought under control and the pattern of abuse broadened to include other drugs.

Among the drugs that have been substituted for fentanyl are sufentanil (Tobin et al., 1988a) and, reportedly, carfentanil and alfentanil. Each of these drugs is more potent than fentanyl and they also cross-react poorly in

the fentanyl assay that has been commercially available. For these reasons abuse of sufentanil was reportedly common in the Western United States, and the possibility of abuse of alfentanil and carfentanil also exists.

Using a forerunner of this sufentanil assay (Tobin et al., 1988a), we detected and controlled a pattern of sufentanil abuse in the Western United States. In at least one state, the horsemen were openly bragging about their use of sufentanil and the inability of racing chemists to detect this drug. Responding to a request for assistance, we used both our fentanyl ELISA and an adaption of our RIA fentanyl assay to detect abuse of this drug. This approach was very successful and led to the detection of about 10 positives for sufentanil in about three hundred samples screened. These events led to the suspension of numerous trainers and, not unexpectedly, to a replacement of the leading trainer at one particular race meet. Since this series of events, however, abuse of sufentanil has been sporadic. This is usually the case in equine drug abuse, where use of the drug declines dramatically once "positives" are called for the drug. New drugs that cannot be detected are then selected for use, depending on their availability and the ability of racing chemists to detect them.

Among the drugs that have a high potential for abuse, and whose use in racing has not yet been detected in racing, are carfentanil and alfentanil. Carfentanil is an analog of fentanyl that is almost as potent as etorphine, the most potent narcotic analgesic commercially available (Tai et al., 1988). Despite the fact that the availability of etorphine is extremely limited, it has been detected in racing horses in Illinois and California, and in Illinois a large number of positives were called for this drug. Since etorphine was a Schedule 1 drug whose use was limited to zoo animal veterinarians, its appearance in a significant number of racetrack samples speaks to the ability of drugs that are manufactured in relatively small

amounts and whose distribution is very strictly controlled to make their way into racing. Even though carfantanil is not readily available, it is, like etorphine, a zoo animal drug, and as such is also likely to make its way into racing.

The development of a useful screening test is a necessary first step for control of the abuse of carfentanil. With the test presented here large numbers of samples can be screened rapidly and effectively for fentanyl, sufentanil and carfentanil. Additionally, if a further measure of sensitivity is required, the samples can be subjected to a simple extraction step and the sensitivity of the test for carfentanil increased. Presumably, also, this step would increase the sensitivity of the test for fentanyl and sufentanil, and in effect allow one to screen for abuse of any of the fentanyls except alfentanil. Such a test would be a very effective screening test for abuse of a cross section of the fentanyls, and makes this sufentanil ELISA a very useful forensic test.

While the confirmation methods for fentanyl and sufentanil are well developed, there is at this time no confirmation method for carfentanil. Although this ELISA test could detect a fentanyl or sufentanil positive, if this test detected carfentanil it would appear simply as an unconfirmable "sufentanil type" positive. This analytical dead end can only be solved by GC/MS research on carfentanil in the horse, since immunoassay positives are familial in nature and require GC/MS confirmation for specific drug identification.

Alfentanil is another fentanyl derivative that has the potential to be diverted to racehorses, since it is approved for use in human medicine in the United States (Shafer et al., 1986). As such, the probability of the appearance of this drug in racing horses is likely higher than for etorphine or carfentanil since the availability of these agents is much more restricted than that of alfentanil. Unfortunately, however, alfentanil does

not cross-react to any significant degree with our fentanyl (Tobin et al., 1988a) or sufentanil antibodies, indicating the necessity for a completely separate test for alfentanil. We therefore derivatized alfentanil, raised antibodies to this drug and developed an ^{125}I RIA to this agent.

Our alfentanil antibody showed what appears to be our standard antibody sensitivity, in that its I-50 for alfentanil was about 1 ng/ml. The antibody was virtually non-reactive with other fentanyls and did not react significantly with sufentanil, carfentanil and fentanyl. This pattern of low cross-reactivity was not unexpected based on the lack of cross-reactivity of antibodies to these drugs with alfentanil. The alfentanil antibody cross-reacted well with two derivatives of alfentanil suggesting that this antibody might cross-react with metabolites of alfentanil in equine blood and urine.

This alfentanil assay readily detected administration of alfentanil in equine plasma, as shown in Figure 13. When two horses were dosed with 500 ug/horse of alfentanil, the initial plasma levels of alfentanil in the order of 400 to 600 picograms/ml were detected. As is typical of the fentanyls, these plasma levels declined rapidly to be virtually undetectable at one hour after dosing. However, the test is sufficiently sensitive to be able to readily detect plasma levels of alfentanil after small doses for up to one hour after administration of this drug.

When we followed the urinary concentrations of alfentanil and its metabolites after IV administration of this drug, we were disappointed in the response that we obtained. The urinary levels of alfentanil equivalents were lower than the plasma levels that we observed in these horses and the urinary concentrations of alfentanil equivalents had returned to baseline by two hours after administration of this drug. This poor urinary detection of alfentanil equivalents suggests that our antibody to alfentanil does not

react well with the urinary metabolites of alfentanil, and only "sees" parent alfentanil, in both blood and urine, for short periods after administration of this drug.

We expect our sufentanil ELISA assay to have uses beyond the application of racetrack drug testing for which it was developed. Sufentanil is a potent drug that is widely used in human medicine and whose use is increasing (Janssen, 1985; Greeley et al., 1987). As such it is a drug that has substantial abuse potential in humans, and therefore a simple and rapid drug screening test for abuse of sufentanil in humans is desirable. In this regard the ability of this test to detect the presence of fentanyl and carfantanil as well as sufentanil is again advantageous. This is particularly so since the concentrations of drug and drug metabolites present in the blood or urine of humans are likely to be much higher than those found in racing horses. Thus screening with the sufentanil ELISA reported here and the alfentanil test should provide adequate screening for most of the fentanyl drugs likely to be abused in horses or humans.

Our ^{125}I RIA for alfentanil may also have applications in studies on the pharmacokinetics of alfentanil in humans. There is at this time no satisfactory assay for alfentanil in human plasma and there are a number of unanswered questions concerning the pharmacokinetics and pharmacodynamics of alfentanil in the human. It has been suggested that the metabolism of alfentanil in the human is polymorphic (Maitre et al., 1987), and evaluation of this possibility in the human requires a sensitive and accurate assay for alfentanil.

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