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Note

Studies on 1-(2-phenethyl)-4-(N-propionylanilino)piperidine (fentanyl) and related compounds

III. Effect of methyl group introduction into fentanyl on sensitivity enhancement in gas chromatography with surface ionization detection

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Fentanyl is an important analgesic used widely in surgical operation, and recently numerous reports have been published on modified fentanyl-related compounds as a "Designer Drug"[1–3]; these substances were characterized by their pharmaceutical activities and by structural modification of control compounds. The amounts of dosage of fentanyl are quite low and the clarification of its use is difficult by reason of its low concentration in blood and urine. There have been some reports on the identification of this drug in blood upon death by overdose[4,5]. In these cases the concentrations of fentanyl in blood, urine and bile were 4–13 ng/ml. The structures of the compounds, α-methylfentanyl, 3-methylfentanyl, *p*-tolylfentanyl, *n*-propylfentanyl, isopropylfentanyl and fentanyl, studied in this report are summarized in Fig. 1.

Some kinds of fentanyl analogues, such as the 3-methyl and α-methyl derivatives, are more potent and the safety margin narrower than that of fentanyl[3,6], so the lethal concentrations of some fentanyls were considered lower than those reported. Furthermore, methods to screen lower concentrations in biological fluids are needed to clarify fentanyl abuse.

The extraction and detection of this kind of compounds in pharmacokinetic studies were performed by complicated methods. Several screening methods of analysis of fentanyls have been reported, such as radioimmunoassay (RIA)[7,8] and gas chromatography with nitrogen sensitive detection (GC–NPD) on a packed column by Woenstenbourghs[9] and also on a capillary column by Watts and Caplan[10].
Surface ionization detection (SID) was introduced in 1985 by Fujii and Arimoto[11] and this method is characterized by its high sensitivity and specificity especially to tertiary amino compounds. We have reported the discrimination of 3- and α-methylfentanyl[12] by several spectroscopic and chromatographic methods and the GC–Fourier trans-

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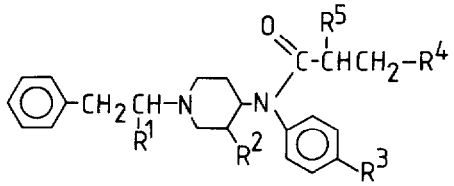

Compound name	R^1	R^2	R^3	R^4	R^5
Fentanyl	H	H	H	H	H
α-Methylfentanyl	CH_3	H	H	H	H
3-Methylfentanyl	H	CH_3	H	H	H
p-Tolylfentanyl	H	H	CH_3	H	H
n-Propylfentanyl	H	H	H	CH_3	H
i-Propylfentanyl	H	H	H	H	CH_3

Fig. 1. Structures of monomethylated fentanyl-related compounds.

form infra-red (FT-IR) analysis of monomethylated fentanyl isomers[13]. In this report, the relationship between the introduction of methyl groups in several positions of fentanyl and the enhancement of the sensitivity of SID is discussed.

EXPERIMENTAL

Materials

Five monomethylated fentanyl-related compounds were prepared by the method of Van Bever *et al.*[6], and fentanyl citrate was obtained from Sankyo (Tokyo, Japan) and examined as its free base.

GC conditions

A Shimadzu GC-14A chromatograph was used with a CBP-1 fused-silica capillary column (film thickness 0.50 μm, 50 m × 0.33 mm I.D.). The GC conditions were: column temperature, 100–320°C (10°C/min); injection temperature, 330°C; carrier gas (helium) flow-rate, 3.5 ml/min; splitless injection. The data obtained were processed with a Shimadzu data processor C-R5A.

SID

The SID conditions were: platium emitter current, 2.2 A; emitter temperature, *ca.* 600°C; ring electrode bias voltage, +200 V with respect to the collector electrode.

Analytical procedures

Each sample (5 pg–100 ng) was injected as a methanol solution into the GC device, and the detection was performed with conventional flame ionization (FID) and SID. The sensitivities of five monomethylated fentanyls and fentanyl itself with these detectors were compared using the peak areas calculated by the C-R5A.

RESULTS AND DISCUSSION

The sensitivity of SID to fentanyl isomers was higher than that of FID. For

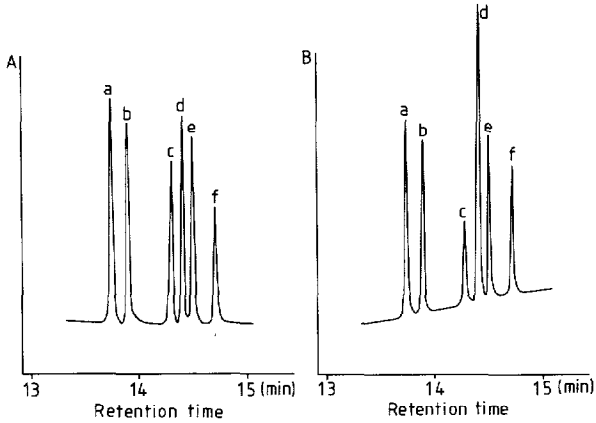

Fig. 2. Gas chromatograms of monomethylated fentanyl isomers by FID (A) and SID (B). Peaks: a = isopropylfentanyl; b = fentanyl; c = 3-methylfentanyl; d = α-methylfentanyl; e = *n*-propylfentanyl; f = *p*-tolylfentanyl.

example, fentanyl was detectable at about 5 pg per injection; on the other hand, by FID analysis, the detection limit was about 10 ng per injection (signal-to-noise ratio, S/N > 10).

The sensitivities of the fentanyls were enhanced unequally. Fig. 2 shows typical chromatograms of fentanyl and five monomethylated fentanyl isomers obtained by using FID (A) and SID (B). For SID, 100 pg of each compound were injected as a methanol solution, and for FID, 100 ng of each.

The calculated peak areas and the sensitivity enhancement relative to fentanyl are summarized in Table I. About 300–1000 times greater sensitivity was observed with SID compared to FID.

There were differences in the sensitivity enhancement of these compounds using SID, especially in the case of α-methyl- and 3-methylfentanyl. Introduction of the methyl group in the α-position of the N-phenethyl group (α-methylfentanyl) was more effective for the enhancement of sensitivity than with other fentanyls. However, introduction of a methyl group into the 3-position of the piperidine ring (3-methyl-

TABLE I

SENSITIVITY ENHANCEMENT RATIOS OF MONOMETHYLATED FENTANYL ISOMERS TO FENTANYL

Compound	*Peak area*		*Sensitivity enhancement*	*Ratio to enhancement of fentanyl*
	FID (counts/0.1 μg)	*SID (counts/0.1 ng)*		
Fentanyl	11457 ± 382	7013 ± 107	612.1	1.00
α-Methylfentanyl	11125 ± 431	11376 ± 365	1022.6	1.67
3-Methylfentanyl	8895 ± 225	2462 ± 82	297.0	0.44
p-Tolylfentanyl	7124 ± 296	5082 ± 116	713.4	1.17
n-Propylfentanyl	9737 ± 183	6288 ± 183	645.8	1.06
Isopropylfentanyl	13140 ± 516	8256 ± 203	628.3	1.03

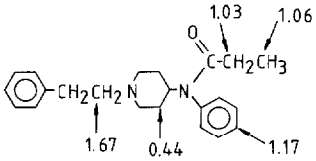

Fig. 3. Sensitivity enhancement induced by methyl group introduction into fentanyl.

fentanyl) reduced the enhancement of sensitivity compared with that for fentanyl. Introduction into the propionyl side chain (*n*-propyl- and isopropylfentanyl) and the *para* position of the anilino group (*p*-tolylfentanyl) resulted in little difference compared with fentanyl.

This phenomena can be explained as follows. In the case of α-methylfentanyl, the electron donating effect of the α-methyl group makes the surface ionization easier than with the other compounds. On the contrary, surface ionization of 3-methylfentanyl was not easy, and this phenomena was presumed to be caused by its steric effect. Since other fentanyl-related compounds, *n*-propyl-, isopropyl- and *p*-tolylfentanyl, did not have these electron donating and/or steric effects, their sensitivity enhancement was nearly equal to that of fentanyl.

The sensitivity enhancement compared to fentanyl is summarized in Fig. 3.

From these results, the important site of surface ionization of fentanyl and related compounds was assumed to be the 1-position of the piperidine ring.

In conclusion, GC–SID was highly sensitive and specific and would be suitable for the detection of these compounds and of use in forensic science. The method has been applied to biological samples, such as blood, and the results will be the subject of a subsequent report.

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