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Studies on 1-(2-phenethyl)-4-(*N*-propionylanilino)piperidine (fentanyl) and related compounds
VII. Quantification of α-methylfentanyl metabolites excreted in rat urine

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ABSTRACT

The use of chemically modified controlled drugs, called designer drugs, is widespread internationally. In the 1980s, the dominant drugs of abuse were modifications of fentanyl formed by methylation of both the α-position of its phenethyl group (α-methylfentanyl) and the 3-position of its piperidine ring (3-methylfentanyl). Numerous analytical methods for fentanyl and its analogues, and many studies of its metabolism and major metabolites, have been reported. However, minor metabolites that reflected injection of the original compound were not included in these studies. Recently, structures of four novel and minor metabolites that reflect α-methylfentanyl have been reported. This study reports excretion amounts of these compounds for 96 h following peroral injection to rats of 3 mg/day and urine collection every 24 h. Major metabolites were the same as for fentanyl, with approximately 24% of α-methylfentanyl excreted as nor-fentanyl and 15% as ω, ω-1 hydroxypropiony nor-fentanyl up to 72 h post-injection. The novel metabolites were completely excreted within 48 h of injection and composed 2–3% of the total metabolite pool. The major metabolite nor-fentanyl was detected up to 72 h after injection.

1. Introduction

Morphine, cannabis and cocaine are classically abused controlled drugs. In addition to these, the past two or three decades have seen the development of chemically synthesized modifications of controlled drugs, called designer drugs. The use of designer drugs has become widespread internationally. Of these compounds, 3,4-methylenedioxy methamphetamine and its analogues have the highest rates of abuse. However, recently the use of tryptamine analogues has spread in Japan. New designer drugs are continually developed around the world in clandestine laboratories. In the 1980s in the United States, the most abused designer drugs were fentanyl analogues [1–5]. The analogues most popular as designer drugs involved methylation of the α-position of phenethyl group bound to the 1-position of piperidine ring (α-methylfentanyl) and the 3-position of the piperidine ring (3-methylfentanyl) [4–6]. Consequently, numerous analytical methods for fentanyl analogues have been reported including; infrared spectroscopy [7,8], gas chromatography–mass spectrometry (GC–MS) [8,9], and nuclear magnetic resonance spectrometry (NMR) [8]. Furthermore, structure-analgesic activities of these analogues have also been reported [10–14]. Numerous studies have investigated the metabolism and major metabolites of fentanyl [15–20]. However, reports concerning fentanyl analogues are limited [21]. This study investigated what metabolites existed that could indicate injection of α-methylfentanyl (**1**). The structures of minor novel metabolites were reported previously, but quantification of these metabolites excreted in rat urine was not discussed [22].

In this report, ten rats were injected with 3 mg/kg of α-methylfentanyl, urine was collected every 24 h up to 96 h post-injection. Urinary excretion of both major and minor metabolites was investigated, focusing on details of those novel minor metabolites that reflected the structure of original compound, α-methylfentanyl. Four minor metabolites involved some hydroxy modification of the aromatic rings and propionyl group in the α-methylfentanyl structure. The first one was of the phenethyl group (*p*-aromatic hydroxy α-methylfentanyl (**2**), while the second and third metabolites were of the ω or ω-1 position of hydroxylated (ω, ω-1 hydroxypropionyl α-methylfentanyl, **3** and **4**) ones. A fourth novel minor metabolite included hydroxy modification of both the *p*-position of aromatic ring of the phenethyl group and ω position of propionyl group hydroxylated

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(*p*-aromatic hydroxyl and ω hydroxypropionyl α-methylfentanyl, **5**). These novel minor metabolites were identified by comparison of compounds synthesized [22]. The major metabolites of α-methylfentanyl were nor-fentanyl (**6**) and ω, ω-1 hydroxypropionyl nor-fentanyl (**7** and **8**); these contributed about 24, 7.3 and 6.8%, respectively, of the metabolized and also excreted α-methylfentanyl.

Twenty-four hours after peroral injection of α-methylfentanyl, nor-fentanyl was excreted dominantly and ω, ω-1 hydroxypropionyl nor-fentanyl were excreted as major metabolites. The *p*-aromatic hydroxy and ω or ω-1 hydroxypropiony α-methylfentanyls were detected in urine 24 h after injection, with *p*-aromatic hydroxy α-methylfentanyl detected until 48 h post-injection. The structures of α-methylfentanyl (**1**), the examined metabolites of α-methylfentanyl (from **2** to **8**) excreted in rat urine and the presumed metabolic pathway of α-methylfentanyl are shown in Fig. 1. This experiment was conducted under permission from the Ethical Committee of Banyu Pharmaceutical Co. Ltd. (Tokyo, Japan).

2. Materials and methods

2.1. Synthesis of α-methylfentanyl and its metabolites

α-Methylfentanyl was synthesized according to the previously reported method [6], its metabolites found in rat urine were also synthesized by a method described earlier [6,9,22–25]. These authentic metabolites were utilized for constructing calibration curves for quantitative determination of metabolites excreted in rat urine. The metabolic pathways for α-methylfentanyl have been reported beforehand [22], including both major (large arrows) and minor metabolic pathways (dotted arrows) in Fig. 1.

2.2. Chemicals

Trifluoroacetyl anhydride was purchased from Tokyo Kasei Industry Co. Ltd. (Tokyo, Japan). The starting compound for synthesis of all metabolites, 1-carbethoxy-4-piperidone, was purchased from Aldrich Co. Inc. (St. Louis, MO, USA). Enzymatic digestion of glucuronized metabolites was conducted using β-glucuronidase purchased from Sigma Co. Inc. (Milwaukee, WI, USA). All other reagents obtained were analytical grade. Water used during the experiment was Milli-Q grade prepared with the Milli-Q system (Nippon Millipore, Tokyo, Japan).

2.3. Animal, injection, urine collection and enzymatic digestion

Ten male Wister rats (120–150 g) were used in this study. They were injected with ca.1.5 ml of a 3% α-methylfentanyl oxalic acid salt aqueous solution. The solution was administrated perorally. This corresponded to a 3 mg α-methylfentanyl dosage, the LD_{50} values for this dosage amount had been reported previously [14] and were taken into consideration. Urine was collected from each rat every 24 h and the individual samples combined. Sampling was conducted until 96 h post-injection. The urine collected (combined approximately 30 ml) each time (0–24, 24–48, 48–72 and 72–96 h), was pH adjusted to 5.0 with 1 M acetic acid (approximately 10 ml) and 100 μg of fentanyl was added as an internal standard. These urine samples were treated with β-glucuronidase (500 units/ml, 37 °C, 12 h) for enzymatic digestion of glucuronides of

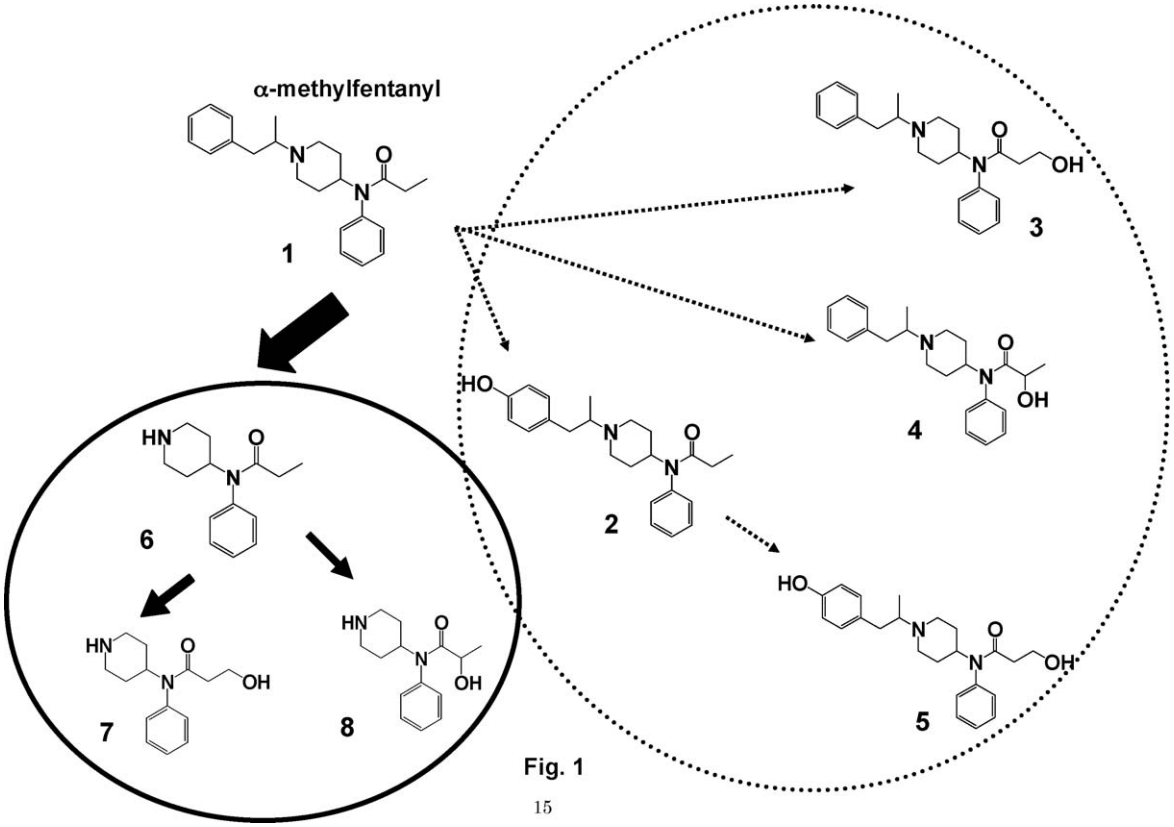

Fig. 1. The structures of fentanyl and its analogues: **1**, α-methylfentanyl; **2**, *p*-aromatic hydroxy α-methylfentanyl; **3**, ω hydroxypropionyl α-methylfentanyl; **4**, ω-1 hydroxypropionyl α-methylfentanyl; **5**, *p*-aromatic hydroxy and ω hydroxypropionyl α-methylfentanyl; **6**, nor-fentanyl; **7**, ω hydroxypropionyl nor-fentanyl; **8**, ω-1 hydroxypropionyl nor-fentanyl.and metabolites detected in rat urine after peroral injection of α-methylfentanyl oxalate. ○: Major metabolites of α-methylfentanyl. ◌: Minor metabolites that reflect the original structure of α-methylfentanyl metabolic pathways are indicated by bold arrows (major) and dotted arrows (minor).