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Pharmacological profile of a potent, efficacious fentanyl derivative in rhesus monkeys*

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Abstract. The recent synthesis of fentanyl derivatives, some of which appear to have novel profiles of pharmacological effects, has provided compelling evidence that μ opioid efficacy might be altered systematically by modifications in the parent compound fentanyl. In the present study a new 4-(heteroanilido)-piperidine, compound 28, was studied for its effects in rhesus monkeys. In self-administration studies compound 28 maintained rates of lever pressing similar to those maintained by alfentanil; the reinforcing effects of compound 28 were attenuated by the opioid antagonist quadazocine. In drug discrimination studies compound 28 did not substitute for the κ agonist ethylketocyclazocine and did substitute for the μ agonist alfentanil. In morphine-treated subjects discriminating between saline and naltrexone, compound 28 did not substitute for naltrexone; however, in morphine-abstinent subjects compound 28 reversed naltrexone lever responding. Moreover, this discriminative stimulus effect in morphine-abstinent subjects was antagonized by naltrexone and by quadazocine in a manner consistent with μ receptor mediation. Compound 28 also was an effective analgesic in a warm-water, tail-withdrawal procedure and it decreased markedly respiratory function. The analgesic effects as well as the respiratory depressant effects of compound 28 were antagonized by quadazocine. Together, these results show compound 28 to be a potent, efficacious μ agonist of similar potency to alfentanil. Large differences in apparent efficacy at μ receptors between compound 28 and another compound in this series (mirfentanil), clearly demonstrate that, within this chemical family, small chemical changes can confer significant differences in pharmacologic effect.

Key words: Opioid – Drug discrimination – Analgesia – Fentanyl – Rhesus monkey – Competitive antagonism – Respiratory function – Self administration

Bagley and colleagues (Bagley et al. 1989) reported on a novel series of fentanyl derivatives, some of which displayed opioid antagonist actions under some conditions. That study provided the first evidence of opioid antagonist action for a compound in the fentanyl (i.e., 4-[heteroanilido]piperidine) series. One compound in this series, mirfentanil (compound 32 in Bagley et al. 1989), has been studied for its effects in vitro and in vivo and has been shown to have a novel pharmacological profile (Aceto et al. 1990; France et al. 1990b, 1991; Ossipov et al. 1990; Woods et al. 1990). For example, as compared to fentanyl, mirfentanil appears to have relatively low efficacy at μ opioid receptors, exerting agonist actions under some conditions and antagonist actions under other conditions. The limited opioid efficacy of mirfentanil was evident under a variety of conditions in rodents and in primates.

Other effects of mirfentanil appear to differ qualitatively among species. For example, there was no evidence of nonopioid actions for mirfentanil in rodents (Bagley et al. 1989), whereas in rhesus monkeys mirfentanil had analgesic effects that did not appear to be mediated by opioid receptors (France et al. 1991). The mechanism of this nonopioid analgesic action for mirfentanil has not been established; moreover, it is not yet clear why some of the effects of mirfentanil are different among rodents, pigeons and primates. Nevertheless, it appears as though further studies on this chemical series might be particularly useful for characterizing the structural requirements that contribute to differences in opioid efficacy.

A second compound in this chemical series, compound 28 (Fig. 1), also appeared to have a profile of pharmacological effects in rodents that was different from the parent compound fentanyl (Bagley et al. 1989). For example, in

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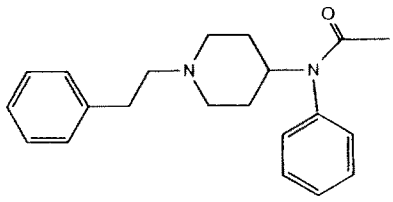

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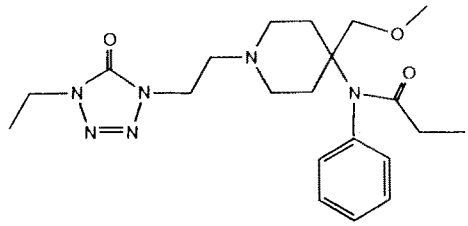
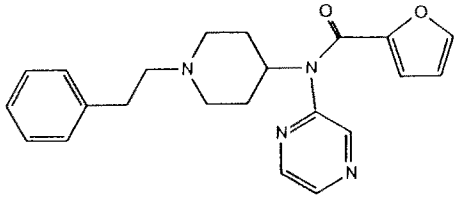
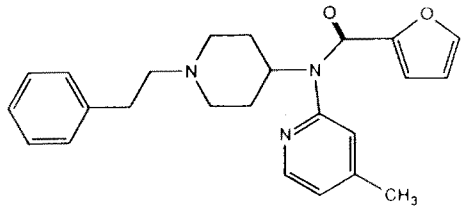


ALFENTANIL



MIRFENTANIL



COMPOUND 28

Fig. 1. Structures of fentanyl, alfentanil, mirfentanil and compound 28

rabbits compound 28 antagonized both the analgesic effects and the respiratory depressant effects of morphine. When studied for agonist actions, compound 28 was effective in some (rabbit tooth pulp, rat tail flick) but not all (rat hot plate, mouse hot plate) analgesia assays. Together these data suggested that, like mirfentanil, compound 28 might have relatively low efficacy as an opioid agonist, and therefore might be a novel opioid with therapeutic potential. As with mirfentanil, there was no suggestion from rodent studies that compound 28 might have nonopioid actions.

The purpose of the present study was to characterize compound 28 in vivo under experimental conditions that have been used extensively for assessing opioid agonists and antagonists, including another fentanyl derivative, mirfentanil (e.g., France et al. 1990b). The results of the present studies demonstrate that, in contrast to the low efficacy opioid actions and nonopioid analgesic actions reported previously for mirfentanil, compound 28 is a highly efficacious, μ-selective opioid with no apparent nonopioid actions in rhesus monkeys.

Materials and methods

Subjects

Seventeen adult male and female rhesus monkeys (*Macaca mulatta*) were housed individually with free access to water. Subjects that discriminated between saline and either alfentanil or ethylketocyclazocine (EKC) were maintained at 85% of their free-feeding body weights by food received in experimental sessions and post-session feeding (Purina Monkey Chow) in the home cage; other subjects had free access to Purina Monkey Chow. All subjects received fresh fruit several times per week. In addition, subjects in the self-administration study had chronic, indwelling intravenous (IV) catheters; subjects discriminating between saline and naltrexone were treated daily with morphine. All subjects used in these studies had previously received various opioid agonists and antagonists.

Apparatus

For self-administration studies, subjects were housed in stainless steel cages measuring 83 × 76 × 51 cm (Woods 1980; Winger et al. 1989); in each cage was a stainless steel panel (15.4 × 15.4 cm) equipped with two response levers and three stimulus lights. A tubular stainless steel harness was connected to a hollow, jointed restraining arm that carried the cannula from the exit site at the midscapular region to the rear of the cage; the catheter was connected to an infusion pump (Watson-Marlow, Co., Model MHRK 55, Falmouth, UK) located behind the cage.

For analgesia studies, drug discrimination studies, and studies on respiratory function, rhesus monkeys were seated in primate chairs that provided minimal restraint at the neck and waist. During drug discrimination and respiration studies the chairs were located in a sound-attenuating, ventilated chamber. For drug discrimination studies two response levers and a food receptacle were located within reach of a seated monkey. An array of stimulus lights located above the response levers indicated schedule conditions (see Procedure). In addition, some chambers were equipped with a pair of shoes containing brass electrodes; electric shock could be delivered to the electrodes by an a.c. shock generator located in an adjacent room. Experimental events were controlled and data recorded with IBM PCjr microprocessors.

Thermos bottles containing 40, 50, or 55° C water were used to study tail withdrawal latencies. A pushbutton switch was connected to a microprocessor used to measure and record latencies.

A closed-chamber head plethysmograph was placed over the subject's head for studies of respiratory function. Plastic neck plates, several rubber dams, and sealant were used to reduce gas leakage from the helmet. Air or 5% CO_2 in air was pumped into the plethysmograph and removed by a vacuum pump (10 l/min). A pressure transducer detected changes in air flow resulting from inspiration and expiration; with a microprocessor pressure changes were translated analog-to-digital and transformed according to known standards to frequency of respiration (f; inspirations/min) and volume of respiration (V_T; tidal volume).

Procedure

Self administration studies. Procedures used to assess positive reinforcing effects of opioids have been described previously (Winger

et al. 1989). Experimental sessions were conducted twice daily with each session comprised of four, discrete components. During each of the four components a red stimulus light was illuminated and IV infusions (drug or vehicle) were available under a fixed ratio (FR) 30 schedule. Each IV infusion was followed by a 45-s timeout during which stimulus lights were extinguished and lever presses had no programmed consequence. Components were separated by a 10-min timeout. A component ended after 25 min or 20 infusions, whichever occurred first; thus, the maximum session length was 130 min [(4 × 25-min component) + (3 × 10-min timeout)]. Responses on a second lever had no programmed consequence.

A different dose of drug was available in each of the four components. The order in which doses were presented within a session was either ascending, descending or mixed (e.g., 0.001, 0.01, 0.00032, 0.0032 mg/kg/injection); the presentation of different dose orders varied randomly among sessions with the same dose order never presented for more than two consecutive sessions. Variations in the dose administered corresponded to changes in the infusion duration for a fixed concentration of drug (e.g., 5-s infusion = 0.0001 mg/kg/injection of alfentanil; 16.7-s infusion = 0.00032 mg/kg/injection of alfentanil). Drug-maintained lever pressing was considered stable and adequate for testing when the rate of lever pressing was dose-related for alfentanil, when at least one dose of alfentanil maintained response rates of at least one response per second, and when the substitution of saline resulted in response rates less than 0.5 responses per second. Compound 28 was studied from doses that produced responding similar to that produced by saline substitution to doses that produced rates of lever pressing ≥ 1 response per second. In other studies, a single IV infusion of the opioid antagonist quadazocine was administered 30 min prior to the beginning of a dose-effect determination with compound 28.

Discrimination studies. Discriminative stimulus effects of compound 28 were assessed in three, separate groups of rhesus monkeys. One group of monkeys discriminated between 0.0056 mg/kg alfentanil and saline; a second group of monkeys discriminated between 0.0032 mg/kg EKC and saline; a third group of monkeys was treated daily with morphine (3.2 mg/kg/day) 3 h prior to experimental sessions and discriminated between 0.01 mg/kg naltrexone and saline. Subjects in the alfentanil and EKC groups responded under FR schedules of food presentation and subjects in the naltrexone discrimination group responded under an FR schedule of stimulus-shock termination.

Daily training sessions consisted of several (1–6) discrete, 15-min cycles with each cycle comprised of a 10-min timeout, during which lever presses had no programmed consequence, and a 5-min response period, during which stimulus lights were illuminated and a schedule of food presentation (alfentanil and EKC groups) or stimulus-shock termination (naltrexone group) was in effect. Under the food schedule subjects could receive a food pellet (300 mg banana-flavored; P.J. Noyes Co., Lancaster, NH) by making 20 (alfentanil group) or 30 (EKC group) consecutive responses on the lever designated correct according to the injection given during the timeout of that cycle (left lever, saline; right lever, alfentanil or EKC). Under the stimulus-shock termination schedule subjects could postpone scheduled shocks for 30 s and terminate a shock-associated visual stimulus by responding 5 times consecutively on the lever designated correct according to the injection given during the timeout of that cycle (left lever, saline; right lever, naltrexone). Under the schedule of stimulus-shock termination, failure to satisfy the response requirement within 15 s resulted in the delivery of a brief electric shock. For subjects responding under the food presentation schedule, stimulus lights were extinguished after 5 min or ten food presentations, whichever occurred first. For subjects responding under the stimulus-shock termination schedule, stimulus lights were extinguished after 5 min or the delivery of four shocks, whichever occurred first. For all subjects the interinjection interval was 15 min and, during training sessions, responses on the incorrect lever reset the response requirement on the correct lever. For some training sessions the cycle during which drug was administered was preceded by one or more saline-injection cycles during which only responding on the left lever resulted in food delivery or postponement of the shock schedule. For other training sessions saline was administered during the timeout of all cycles.

Test sessions were identical to training sessions, except that subjects could receive food or postpone scheduled shocks by satisfying the FR requirement on either lever, and increasing doses of drug were administered during the timeout of consecutive cycles such that the cumulative dose increased by 0.5 or 0.25 log units per cycle. Compound 28 was studied up to doses that either substituted completely for a training drug (alfentanil, EKC or naltrexone) or to doses that decreased substantially rates of lever pressing. Other studies in morphine-treated monkeys discriminating between naltrexone and saline further characterized the opioid agonist actions of compound 28. Specifically, compound 28 was studied in morphine-abstinent (i.e., saline-treated) monkeys for its ability to attenuate naltrexone lever responding. Because compound 28 attenuated naltrexone-lever responding in morphine-abstinent subjects (see Results), other studies examined possible antagonism of this effect by the opioid antagonists naltrexone and quadazocine. For antagonism studies, subjects received saline 3 h prior to an experimental session during which a dose of opioid antagonist was administered on the first cycle and increasing doses of compound 28 were administered on subsequent cycles.

Analgesia studies. Procedures used to assess analgesic effects in rhesus monkeys have been described previously (Dykstra and Woods 1986). Experimental sessions consisted of several discrete 30-min components with each component comprised of a 20-min timeout, during which subjects were not handled or disturbed, and a 10-min assessment period during which the latency for subjects to remove their tails from warm water was determined for 40, 50 and 55° C water. For tail withdrawal latencies the lower 10–15 cm of the shaved tail was immersed in a thermos containing water; if the subject failed to remove its tail within 20 s the experimenter removed the thermos and a latency of 20 s was recorded for that subject. Increasing doses of drug were administered during the first minute of consecutive timeouts with the cumulative dose increasing by 0.5 log units per cycle. To determine whether analgesic effects of compound 28 were mediated by opioid receptors, a dose-effect determination was repeated for compound 28 beginning 30 min after SC administration of the opioid antagonist quadazocine.

Respiration studies. Procedures for assessing effects of drugs on respiratory function in rhesus monkeys have been described previously (Howell et al. 1988). Sessions consisted of several discrete, 30-min cycles with each cycle comprised of a 23-min exposure to air followed by a 7-min exposure to 5% CO_2 in air. Respiration [frequency (f) and tidal volume (V_T)] was monitored continuously and data are reported for the last 3 min of exposure to air and the last 3 min of exposure to CO_2. Increasing doses of drug were administered SC during the first minute of consecutive timeouts with the cumulative dose increasing by 0.5 log units per cycle. Compound 28 was studied up to doses that decreased respiratory function to $\leq 60\%$ of control. To determine whether respiratory depressant effects of compound 28 were mediated by opioid receptors, a dose-effect determination was repeated for compound 28 beginning 30 min after SC injection of various doses of quadazocine.

Drugs

The drugs used in these studies were morphine sulfate (Mallinckrodt, Inc., St Louis, MO), naltrexone hydrochloride (Endo Laboratories, Inc., Garden City, NY), alfentanil hydrochloride (Janssen Pharmaceutica, Piscataway, NJ), quadazocine methanesulfonate (WIN 44,441; Sterling-Winthrop, Rensselaer, NY), and ethylketocyclazocine methanesulfonate (EKC; Sterling-Winthrop, Rensselaer, NY). Compound 28 was synthesized by one of the authors (MRS) according to the procedures of Bagley et al. (1989).

Data analyses

Results of drug self-administration studies are rates of lever pressing expressed in responses per second as a function of dose (mg/kg body weight/injection). Results from drug discrimination studies are expressed as the average percentage of responses on the drug lever [% drug responding (%DR)] ± 1 SEM and are plotted as a function of dose. Compound 28 was considered to have substituted for a training compound if it produced an average of ≥ 90% responding on the drug-associated lever. The dose of compound 28 required for 50% effect (A_{50}) in monkeys discriminating between saline and naltrexone was estimated from control (no antagonist) dose-effect curves and from each dose-effect curve determined in the presence of various doses of naltrexone or quadazocine. The log of [dose ratio (A_{50} with antagonist (A')/A_{50} without antagonist (A)) − 1] was plotted as a function of the negative log dose of antagonist (B), i.e., Schild plot (Arunlakshana and Schild 1959). Other analyses were also conducted for compound 28 administered in combination with either naltrexone or quadazocine with the Schild plot slopes constrained to − 1 (Tallarida et al. 1979). Response rate data from the drug discrimination study are expressed as the mean response rate ± 1 SEM and calculated as a percentage of the control (no drug) response rate. Tail withdrawal latencies are expressed as a percentage of the maximum possible effect (20 s) and calculated as: [test latency minus control (no drug) latency] divided by (20 minus control latency) multiplied by 100. Percentages are plotted as a function of dose and represent the average of single determinations in each of four subjects ± 1 SEM. Results from the respiration study are presented as the average f and V_T ± 1 SEM expressed as a percentage of f and V_T under control (no drug) conditions in monkeys breathing air or 5% CO_2 in air.

Results

Self-administration studies

In all three subjects, increases in the dose of compound 28 produced increases in rates of self-administration responding (Fig. 2). Over the dose range studied, maximum response rates were obtained in all of the subjects with a dose of 0.0003 mg/kg of compound 28; larger doses of compound 28 (data not shown) maintained rates of lever pressing less than those maintained by 0.0003 mg/kg. In all three subjects a dose of 0.0003 mg/kg alfentanil produced rates of lever pressing (2.30 ± 0.54 responses per second; data not shown) that exceeded response rates obtained with any dose of compound 28. When saline was substituted for alfentanil rates of responding averaged 0.17 ± 0.06 responses per second.

The positive reinforcing effects of compound 28 were antagonized in a dose-related manner by quadazocine. Pretreatment with a dose of 0.01 mg/kg quadazocine shifted the compound 28 dose-effect curve slightly (< 3 fold) to the right and decreased the maximum obtainable rate of responding. A 10-fold larger dose of quadazocine, 0.1 mg/kg, shifted the compound 28 dose-effect curve an additional 10-fold to the right and also decreased the maximum effect.

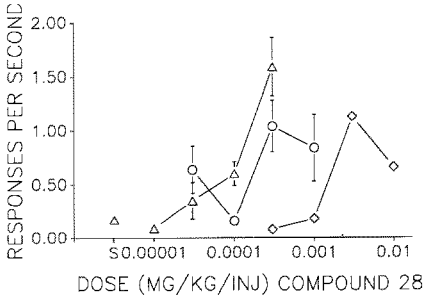


Fig. 2. Self administration of compound 28 under control conditions and in the presence of pretreatment doses of 0.01 or 0.1 mg/kg quadazocine. *Ordinate*: averaged rate of lever pressing in responses per second. With the exception of results obtained after pretreatment with 0.1 mg/kg quadazocine ($n = 2$), each point is the average of three subjects. *Abscissa*: dose of compound 28 in mg/kg. Data point above *S* represents the effects of saline. Quadazocine dose (mg/kg): (△) 0; (○) 0.01; (◇) 0.1

Drug discrimination studies

The administration of increasing doses of alfentanil produced dose-related generalization to the drug-associated lever in subjects discriminating between saline and alfentanil with complete generalization (i.e., ≥ 90% responding on the drug lever) occurring with doses of alfentanil larger than 0.0056 mg/kg (diamonds, upper left panel, Fig. 3). Compound 28 produced a dose-related switch in responding from the saline lever to the alfentanil lever with complete generalization occurring with doses of compound 28 larger than 0.001 mg/kg (triangles, upper left panel, Fig. 3). Alfentanil and compound 28 produced only small decreases in response rate at doses which occasioned responding on the drug lever (lower left panel).

Administration of increasing doses of EKC produced dose-related generalization to the drug-associated lever in subjects discriminating between saline and EKC with complete generalization occurring with doses of EKC larger than 0.001 mg/kg (squares, upper right panel, Fig. 3). In contrast to the complete substitution obtained with compound 28 in monkeys discriminating between saline and alfentanil, compound 28 produced only saline-lever responding, up to a dose of compound 28 that eliminated responding (0.01 mg/kg), in monkeys discriminating between saline and EKC. Monkeys discriminating saline from EKC appeared to be more sensitive to the rate-decreasing effects of compound 28 as compared to monkeys discriminating saline from alfentanil (compare triangles, lower panels, Fig. 3).

In monkeys receiving daily injections of morphine 3 h prior to experimental sessions, increasing doses of naltrexone produced a dose-related switch in responding from the saline lever to the naltrexone lever with complete generalization occurring with doses of naltrexone larger than 0.0032 mg/kg (circles, upper left panel, Fig. 4). Up to a dose that eliminated responding (0.32 mg/kg), compound 28 failed to produce any responding on the naltrexone-associated lever in morphine-treated monkeys (triangles, left panels, Fig. 4). Doses of compound 28 that eliminated responding maintained by food presentation in monkeys discriminating between saline and EKC had no effect on responding maintained by stimulus-shock termination in morphine-treated monkeys discriminating

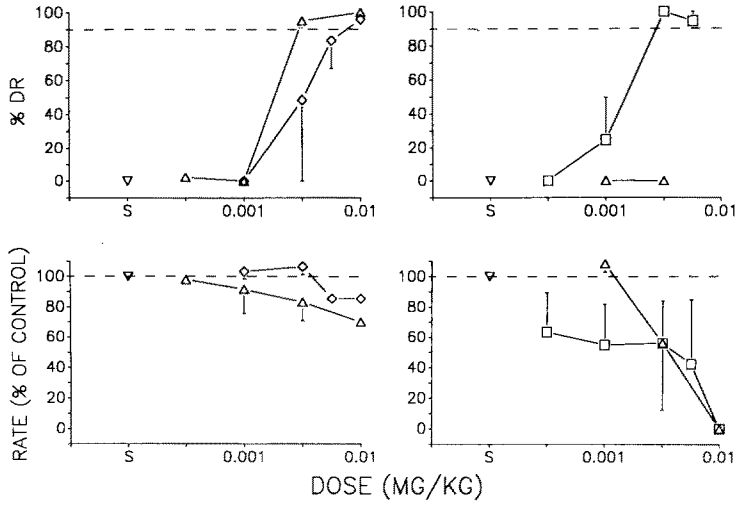


Fig. 3. Discriminative stimulus effects and response rate effects of compound 28 in separate groups of rhesus monkeys discriminating between saline and either 0.0056 mg/kg alfentanil ($n = 2$; *left panels*) or 0.0032 mg/kg EKC ($n = 2$; *right panels*). *Ordinates*: *upper panels*, percentage of responses on the drug lever [drug responding (%DR)] $\pm$ 1 SEM; *lower panels*, averaged rate of lever pressing on both levers expressed as a percentage of response rates under control (no drug) conditions. *Abscissae*: dose in mg/kg body weight. Data points above *S* represent the effects of a SC injection of saline. (◇) Alfentanil; (△) compound 28; (□) ethylketocyclazocine

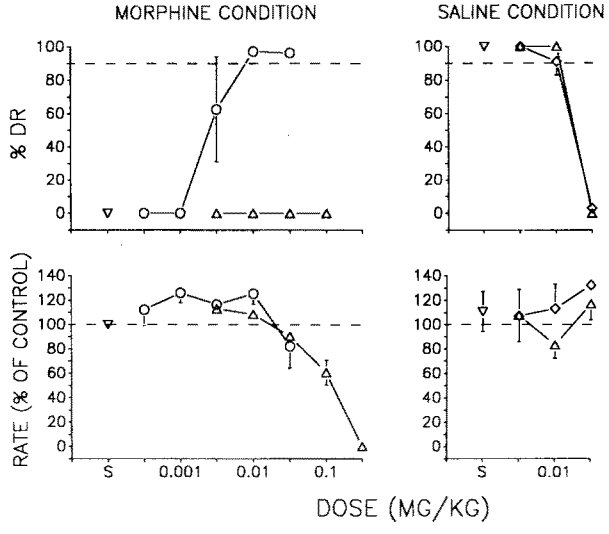


Fig. 4. Discriminative stimulus effects and response rate effects of compound 28 in three morphine-treated monkeys discriminating between saline and 0.01 mg/kg of naltrexone: *left panel*, effects of compound 28 and naltrexone in subjects that received 3.2 mg/kg morphine 3 h earlier (i.e., morphine condition); *right panel*, effects of compound 28 and alfentanil in subjects that received morphine 27 h earlier and saline 3 h earlier (i.e., saline condition). Data points above *S* indicate the effects of saline under each of the two treatment conditions. See Fig. 3 for other details (◇) Alfentanil; (△) compound 28; (○) naltrexone

between saline and naltrexone (triangles, lower right panel of Fig. 3 and lower left panel of Fig. 4, respectively).

When saline was substituted for the daily injection of morphine, subjects responded exclusively on the naltrexone lever (point above *S*, upper right panel, Fig. 4). Alfentanil attenuated the naltrexone-lever responding that occurred in saline-treated (morphine-abstinent) monkeys with complete attenuation ($\leq$ 10% responding on the naltrexone lever) occurring with a dose of 0.032 mg/kg alfentanil. Compound 28 also reversed naltrexone lever responding and was equipotent to alfentanil in this regard (compare diamonds and triangles, upper right panel, Fig. 4).

The alfentanil-like discriminative stimulus effects of compound 28 observed in morphine-abstinent (saline-treated) monkeys (i.e., reversal of naltrexone lever responding) was antagonized by the opioid antagonists naltrexone and quadazocine (Fig. 5). Under control conditions, a dose of 0.032 mg/kg of compound 28 reversed completely naltrexone lever responding (triangles, upper panels, Fig. 5). In contrast, when subjects had received either 0.032 mg/kg naltrexone (squares, upper left panel) or 0.1 mg/kg quadazocine (diamonds, upper right panel), a dose of 1.0 mg/kg of compound 28 was required to reverse completely naltrexone lever responding. From dose-effect curves determined for compound 28 in combination with naltrexone or quadazocine, Schild plots were constructed (lower panels, Fig. 5) and apparent affinity estimates were calculated using pA_2 analyses. The pA_2 values for naltrexone and quadazocine were: 8.26 and 7.85, respectively, with unconstrained slopes; 8.41 and 7.87, respectively, when slopes were constrained to -1. Apparent affinities for naltrexone and quadazocine in combination with compound 28 were similar to apparent affinities for the same antagonists administered in combination with other agonists (Table 1).

Analgesia studies

Under control conditions the average latency for monkeys to remove their tails from warm water was 20 $\pm$ 0, 0.86 $\pm$ 0.08, and 0.65 $\pm$ 0.09 s for 40, 50 and 55° C, respectively. Compound 28 increased in a dose-related manner the latency for monkeys to remove their tails from warm (50 or 55° C) water (Fig. 6), producing latencies that were $\geq$ 90% of the maximum possible effect (i.e., 20-s latencies) at a dose of 0.178 mg/kg (50° C, left panel; 55° C, right panel). The analgesic effects of compound 28 were antagonized in a dose-related manner by quadazocine (Fig. 6). The analgesia dose-effect curves for compound 28 were shifted 3-fold to the right after pretreatment with 0.1 mg/kg quadazocine; pretreatment with a dose of

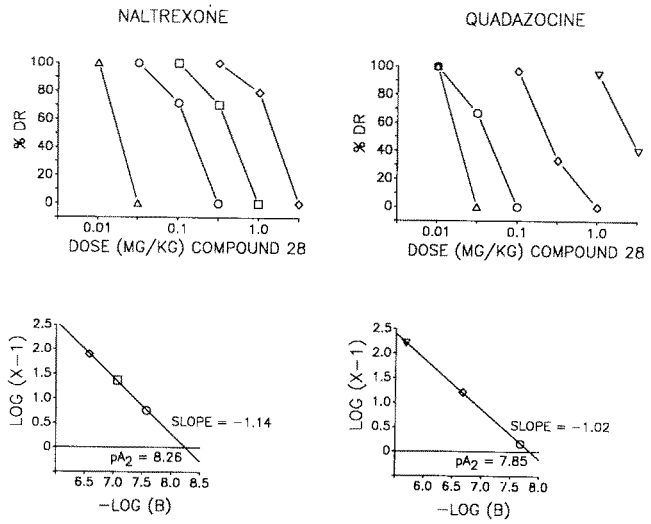


Fig. 5. Dose-effect curves for compound 28 administered alone and in combination with various doses of either naltrexone (*left panel*) or quadazocine (*right panel*) in three monkeys discriminating between saline and 0.01 mg/kg naltrexone. Subjects received 3.2 mg/kg morphine 27 h earlier and saline 3 h earlier. Antagonists were administered 15 min prior to the first injection of compound 28. See Figs 3 and 4 for other details. Antagonist dose (mg/kg): (△) 0; (○) 0.01; (□) 0.032; (◇) 0.1; (▽) 1.0

1.0 mg/kg quadazocine resulted in antagonism that was not surmounted by compound 28 up to the maximum dose that could be studied, 1.78 mg/kg.

Respiration studies

Table 2 shows the average frequency (f) and tidal volume (V_T) of respiration for three monkeys breathing normal air or 5% CO_2 in air. Exposure to CO_2 increased the average f and V_T to 142.8 ± 2.8% and 120.9 ± 6.5% of control, respectively. Compound 28 decreased f and V_T in a dose-related manner in subjects breathing air and in subjects breathing 5% CO_2 in air (Fig. 7). A dose of 0.001 mg/kg of compound 28 had little or no effect on f or V_T with larger doses producing progressively larger decreases in both measures of respiratory function. At the largest dose studied, 0.1 mg/kg, f and V_T were decreased to < 50% of control under both conditions (air and CO_2).

The respiratory depressant effects of compound 28 were attenuated in a dose-related manner by quadazocine (Fig. 8). Pretreatment with a dose of 0.01 mg/kg quadazocine shifted the f and V_T dose-effect curves 2–3 fold to the right of control dose-effect curves. Larger doses of quadazocine produced further shifts to the right in the compound 28 dose-effect curves. For example, under control conditions a dose of 0.1 mg/kg of compound 28 decreased V_T to < 50% of control; in the presence of 1.0 mg/kg quadazocine V_T was ≥ 85% of control up to a dose of 1.0 mg/kg of compound 28 (right panel, Fig. 8). Qualitatively similar effects were obtained for quadazocine in combination with compound 28 in monkeys breathing air (data not shown).

Table 1. Apparent affinity estimates for naltrexone and quadazocine in combination with compound 28, alfentanil and morphine in a drug discrimination procedure

Agonist	Naltrexone pA_2 (slope)	Quadazocine pA_2 (slope)
Compound 28	8.26 (− 1.14)[a]	7.85 (− 1.02)
Alfentanil[b]	8.69 (− 0.97)	7.55 (− 1.14)
Morphine[b]	8.32 (− 1.04)	–[c]

[a] Apparent affinity using the method of Arunlakshana and Schild (1959)
[b] From France et al. (1990a)
[c] Not studied

Table 2. Control (no drug) frequency (f) and tidal volume (V_T) of respiration for monkeys breathing air or 5% CO_2 in air

Subject	Air		5% CO_2	
	f[a]	V_T[b]	f[a]	V_T[b]
RE	34	56.5	48	63.5
SA	23	94.9	32	126.8
EL	27	77.2	40	90.0

[a] Frequency of respiration in breaths/min
[b] Volume of respiration in ml/inspiration

Discussion

Several clinically useful compounds have been developed from the 4-[heteroanilido]piperidine series, including fentanyl, sufentanil, and alfentanil. All three of these compounds share many effects with morphine, including profound respiratory depressant effects at large doses. The

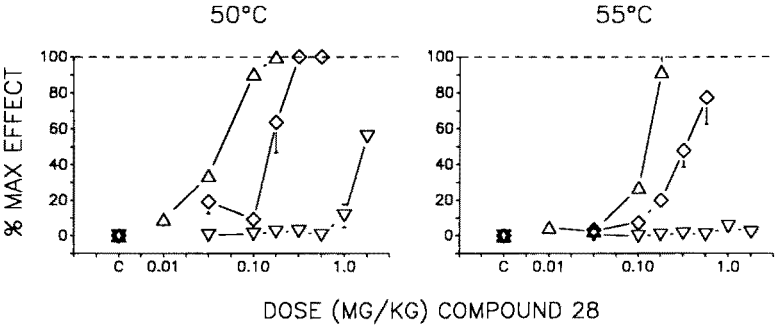


Fig. 6. Effects of compound 28 on tail withdrawal latencies under control conditions (*triangles*) and in the presence of 0.1 (◇) or 1.0 mg/kg (▽) quadazocine from 50 and 55°C water (*left* and *right panels*, respectively). *Ordinates*: average tail withdrawal latencies ± 1 SEM are expressed as a percentage of the maximum possible effect (i.e., 20 s) for four monkeys. *Abscissae*: dose of compound 28 in mg/kg body weight. Data points above *C* represent the effects of saline or quadazocine prior to administration of compound 28

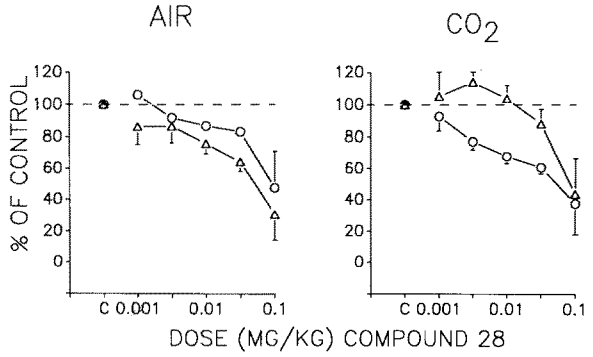


Fig. 7. Effects of compound 28 on respiratory function (f and V_T) in three monkeys breathing air (*left panel*) or breathing 5% CO_2 in air (*right panel*). *Ordinates*: averaged f and V_T expressed as a percentage of f and V_T, respectively, under the corresponding condition (air or CO_2) in the absence of drug (*C*; 100%). *Abscissae*: dose of compound 28 in mg/kg body weight. (○) Frequency (f); (△) tidal volume (V_T)

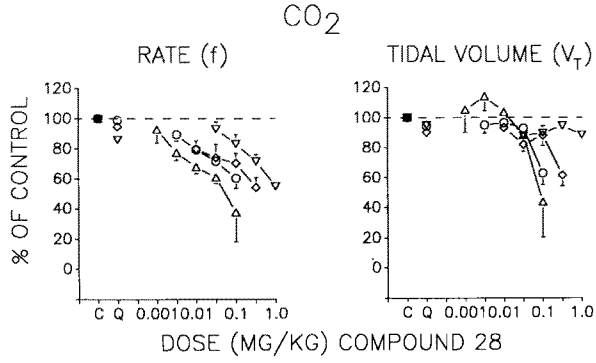


Fig. 8. Antagonism by quadazocine of the respiratory depressant effects of compound 28. Dose-effect curves were determined for compound 28 under control conditions (*triangles*) and beginning 30 min after an acute injection of quadazocine (0.01–1.0 mg/kg). See Fig. 7 for other details. Quadazocine dose (mg/kg): (△) 0; (○) 0.01; (◇) 0.1; (▽) 1.0

novel profile of action reported for mirfentanil (Bagley et al. 1989; France et al. 1991), therefore, was particularly interesting because there had not been any previous report of compounds from the fentanyl series which had opioid antagonist actions. In the present study another fentanyl derivative, compound 28, was studied to see whether it might also exert opioid antagonist or nonopioid agonist actions in rhesus monkeys. However, in contrast to the low efficacy opioid agonism observed for mirfentanil, compound 28 had agonist effects under all experimental conditions. Moreover, all of these agonist effects were antagonized by quadazocine. Thus, compared to mirfentanil, compound 28 appears to have considerably higher efficacy at μ receptors.

The effects of some fentanyl derivatives appear to be quite varied among different species. For example, in rats, rabbits, and mice both mirfentanil and compound 28 had limited opioid agonists actions and, under some conditions, had opioid antagonist actions (Bagley et al. 1989). Second, there was no report of any nonopioid (i.e., naloxone-insensitive) effects for either of these compounds in non-primate species. Mirfentanil also was reported to be more effective than compound 28 in producing analgesic effects in non-primate species. In contrast to results obtained in other species, in rhesus monkeys compound 28 was a potent, efficacious morphine-like agonist with no apparent nonopioid effects. The profile of action obtained with compound 28 in rhesus monkeys was qualitatively the same as that obtained with alfentanil under the same conditions. Together with results obtained in several different species for mirfentanil, results from the current study on compound 28 suggest pharmacological effects of fentanyl derivatives, and perhaps other compounds, in mice, rats or rabbits cannot be assumed to predict their effects in other species.

Sensitivity to rate-decreasing effects of compound 28 varied markedly among different groups of monkeys. For example, morphine-treated monkeys discriminating between saline and naltrexone were 30 times less sensitive to the rate-decreasing effects of compound 28 than monkeys discriminating between saline and EKC. This difference in sensitivity was not simply the result of different schedule conditions between the two groups (food versus stimulus-shock termination) as monkeys discriminating between saline and alfentanil under a food schedule were also less sensitive to the rate-decreasing effects of compound 28. Moreover, doses of compound 28 that were studied for discriminative stimulus effects in morphine-treated monkeys (> 0.01 mg/kg) could not be studied in other experiments because of the profound respiratory-depressant effects of these doses in untreated subjects (see Fig. 8). Thus, cross tolerance to compound 28 was evident only in subjects that frequently received morphine or morphine-like agonists, providing further evidence of the μ agonist mechanism of action for compound 28 in rhesus monkeys.

The selectivity and potency of opioid antagonists in preventing the effects of different opioid agonists has been used widely to differentiate receptor mechanisms. One quantitative method that has been used to analyze behavioral effects of opioids involves apparent affinity estimates for selective antagonists (e.g., Takemori 1974). The apparent affinity estimates for naltrexone were similar regardless of whether the agonist was alfentanil (pA_2 = 8.69; France et al. 1990a), morphine (pA_2 = 8.32; France et al. 1990a) or compound 28 (pA_2 = 8.26; present study); similarly, the pA_2 values for quadazocine were 7.55 in combination with alfentanil (France et al. 1990a) and, in the present study, 7.85 in combination with compound 28. The similarity of pA_2 values obtained for naltrexone and for quadazocine with compound 28, alfentanil and morphine, drugs that vary in absolute potency by more than 170 fold, strongly supports the notion that all three compounds exert their discriminative stimulus effects at the same receptor (i.e., μ). Moreover, the striking similarity among these in vivo apparent affinity estimates further demonstrates the utility of this quantitative approach for differentiating receptor-mediated behavioral effects (Takemori 1974; Bertalmio and Woods 1987; Dykstra et al. 1987).

Although results from the present studies do not provide any direct evidence for a nonopioid effect of compound 28, it is possible that compound 28 might have nonopioid effects that are masked by opioid effects. For

example, decreases in maximum rates of self administration of compound 28 when subjects received a large dose of an opioid antagonist (i.e., quadazocine) might have resulted from an unmasking of a nonopioid, and non-reinforcing, effect of compound 28. Whereas the self administration dose-effect curve for alfentanil, a compound for which nonopioid actions have not been demonstrated, was shifted 30–300 fold to the right by quadazocine with little change in the maximum rate of responding (Bertalmio and Woods 1989; G. Winger, unpublished observation), the self-administration dose-effect curve for mirfentanil, a compound for which nonopioid actions have been demonstrated, first was shifted 3-fold to the right by quadazocine then shifted down with a decrease of more than 50% in the maximum rate of responding (France et al. 1991). Additional studies with compound 28, particularly in the presence of large doses of opioid antagonists, might unmask a mirfentanil-like, nonopioid action for compound 28.

The results obtained in the current study with the fentanyl derivative compound 28 (Bagley et al. 1989) demonstrate that a small modification in the structure of mirfentanil confers strong μ agonism, which appears to either occur in the absence of, or prevent the expression of, the nonopioid actions observed for mirfentanil (France et al. 1991). Further studies on the structure-activity relations of other fentanyl derivatives might provide important insights in to the physicochemical requirements for efficacy at the μ opioid receptor.

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