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Evaluation of 2-Azabicyclo[2.2.2]octane Analogs of 4-Anilidopiperidine Analgesics

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Abstract □ Eight analogs of the fentanyl-type analgesics, in which the piperidine ring is restricted into a boat conformation, were evaluated for analgesic activity. All analogs were less active than fentanyl, but interesting conformational and structural relationships were observed. Results of the study are discussed.

Keyphrases □ Analgesic activity—evaluation of 2-azabicyclo[2.2.2]-octane analogs of 4-anilidopiperidines, mice □ 4-Anilidopiperidines—2-azabicyclo[2.2.2]octane analogs, evaluation for analgesic activity, mice □ Structure–activity relationships—evaluation of 2-azabicyclo[2.2.2]-octane analogs of 4-anilidopiperidines for analgesic activity, mice

Studies designed to clarify the structural and conformational factors influencing the interactions of centrally acting analgesic agents with analgesic receptors have received considerable attention (1), and the recent discovery of the natural opiates has intensified this interest (2). Structural modification of methadone and methadone derivatives led to the development of a new series of agents, the acyclic basic anilides (3), which are represented by diampromide (I). Subsequent development (4) of the 4-anilidopiperidines, represented by fentanyl (II), led to a potent new class of analgesics. Differences in the modes of receptor binding of the various classes have been suggested.

BACKGROUND

The acyclic basic anilides have been proposed to differ from the methadone-type analgesics (5), and the 4-anilidopiperidines apparently differ from the acyclic basic anilides and the 4-phenylpiperidines, although they more closely resemble the former group (6). Differences in the modes of receptor binding between the acyclic basic anilides and the 4-anilidopiperidines have been proposed on the basis of structure–activity relationships and may be due to differences in receptor conformations (6). Based on spectroscopic evidence (7), a conformation of I has been suggested in which, among other features, the protonated basic center lies in close proximity to the anilido nitrogen. The preferred conformation of II has been suggested (6) as that in which the anilido function assumes the equatorial position of the chair form of the piperidine ring. Such a conformation would place the protonated basic center at a greater distance from the anilido nitrogen in II than in I. Additionally, this conformation of II would differ from the preferred conformation of the 4-phenylpiperidines, which has been suggested to be a skew boat conformation (8). However, the possibility that boat-type conformations of II may contribute to receptor interactions has not been tested directly. Analogs of fentanyl in which the flexibility of the piperidine ring has been restricted through incorporation of the tropane nucleus, designed to simulate frozen chair conformations of fentanyl, were reported recently (9). The 3-β-(propanilido) isomers were more potent than the 3-α-(propanilido) isomers and were nearly as potent as fentanyl itself.

The 2-azabicyclo[2.2.2]octane ring system was employed previously to restrict acetylcholine (10) and procaine (11) in *trans-* and *gauche-*conformations. More recently, this ring system was employed to restrict the piperidine ring of prodine-type analgesics in boat conformations (12). This report concerns the pharmacological evaluation of boat conformers of fentanyl-type analgesics. Casy *et al.* (6) reported the effects of substituents on the ring nitrogen atom of 4-anilidopiperidines and noted that analgesic activity increases significantly in the order $CH_3 < CH_2C_6H_5 < CH_2CH_2C_6H_5$. The analogs prepared in this study would permit the evaluation of this effect as well as the effect of positional isomerism in the boat conformers III–X. The synthesis and stereochemical analyses of these analogs were reported previously (13).

EXPERIMENTAL

The analgesic potency of the fentanyl analogs was determined by a modification of the D'Amour–Smith tail-flick method (14) using male albino ICR mice[1], 25–30 g, and an analgesiometer. All mice had their tails blackened with a black magic marker at least 15 min prior to testing to ensure a short, uniform tail-flick response time. The challenge to thermal stimulus occurred 30 min postinjection with the vehicle alone or the respective drugs. A control group of 10 mice was used to establish the baseline response time.

The criterion for analgesia (antinociception) was defined as a tail-flick response time greater than or equal to the mean response time of the

[1] Harlan Industries, Cumberland, Ind.

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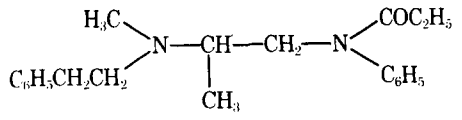

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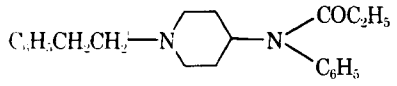

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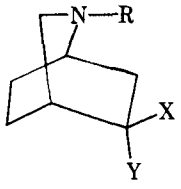
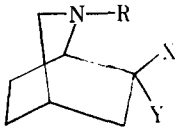


III: R = CH_3, X = $N(C_6H_5)COC_2H_5$, Y = H
IV: R = CH_3, X = H, Y = $N(C_6H_5)COC_2H_5$
V: R = $CH_2CH_2C_6H_5$, X = $N(C_6H_5)COC_2H_5$, Y = H
VI: R = $CH_2CH_2C_6H_5$, X = H, Y = $N(C_6H_5)COC_2H_5$



VII: R = CH_3, X = $N(C_6H_5)COC_2H_5$, Y = H
VIII: R = CH_3, X = H, Y = $N(C_6H_5)COC_2H_5$
IX: R = $CH_2CH_2C_6H_5$, X = $N(C_6H_5)COC_2H_5$, Y = H
X: R = $CH_2CH_2C_6H_5$, X = H, Y = $N(C_6H_5)COC_2H_5$

control group plus two standard deviations of their mean. Groups of eight mice were used for each dose of drug. Morphine sulfate alone and together with fentanyl citrate served as the positive controls. All AD_{50} values and their 95% confidence limits were determined by the method of Litchfield and Wilcoxon (15).

All injections were made intraperitoneally with saline as the vehicle. The drug preparation was injected at 0.01 ml/g. The *N*-methyl analogs (III, IV, VII, and VIII) were administered as the hydrochloride salts, while the *N*-phenethyl analogs (V, VI, IX, and X) were administered as suspensions of free bases in 4% polysorbate–saline because of the difficulty in producing homogeneous solutions at neutral pH in pure saline. Compounds that produced no analgesia at 100 mg/kg were tested further for possible antagonism to the narcotics. For the antagonism study, at least four mice per group were pretreated with 5.0 mg of morphine sulfate/kg or 500 μg of fentanyl citrate/kg 5–10 min before injection of the test compound and subsequent analgesic testing 30 min postinjection.

RESULTS AND DISCUSSION

The results of the analgesic evaluation and antagonism study are summarized in Tables I and II, respectively. Both *N*-methyl-5-propanilido analogs (III and IV) were analgesically inactive at 100 mg/kg. With the *exo*-isomer (III), central nervous system effects were observed clearly. Mice treated at that dose showed decreased motor activity, labored respiration, and tremor. No blockade of the morphine analgesic effect was demonstrated for III and IV, although most of the animals treated with morphine and III (100 mg/kg) failed to produce the Straub tail effect.

Both *N*-methyl-6-propanilido analogs (VII and VIII) showed analgesic activity with AD_{50} values of 60.0 and 71.5 mg/kg, respectively. However, in both cases, no Straub tail effect was produced along with the analgesic effect. Both of these agents also were highly toxic, with LD_{50} values of 62.5 and 91.0 mg/kg, respectively. The animals evidenced severe dyspnea and convulsions at higher doses. In the *exo*-isomer (VII), overlap occurred between the confidence intervals for the AD_{50} and LD_{50}. This overlap was possible since analgesic testing of the survivors at a given dose contributed data to the AD_{50} determination.

Unlike the *N*-methyl derivatives, water-soluble hydrochloride salts of the *N*-phenethyl analogs could not be prepared, suggesting large differences in hydrophobic properties in the two series. Of the four isomeric *N*-phenethyl analogs (V, VI, IX, and X), only X showed activity (AD_{50} value of 43.0 mg/kg). Compound X also had a relatively wider safety margin (therapeutic index > 3) as compared to the two active *N*-methyl-6-propanilido analogs (VII and VIII). Toxicity was observed only when the dosage was raised to 150 mg/kg, resulting in extreme excitation, convulsions, and lethality in two of eight mice. The three analgesically inactive *N*-phenethyl analogs (V, VI, and IX) produced only mild dyspnea and convulsions, and no deaths occurred in all dosage groups tested. These analogs also were unable to antagonize the analgesic activity of fentanyl.

Prior to the utilization of 4% polysorbate 60–saline as a suspending vehicle for the four *N*-phenethyl analogs, suspensions of V, VI, IX, and X in 3% acacia–saline were used for administration *via* the intracerebroventricular route. The intracerebroventricular injections were made after careful removal of the skin from the top of the skull with a razor. Injections were made at a point of 1 mm posterior to the bregma and 1 mm laterally to the right of the midline of the skull (16). Five microliters then was injected (for a 25-g mouse) slowly ~1 mm into the right lateral ventricle.

Analgesia was determined at 20 and 60 min postinjection. While the AD_{50} values of morphine sulfate and fentanyl citrate were 0.21 mg/kg and 14 μg/kg by this administration route, the experiment did not lead to an AD_{50} determination of the analogs due to the limitations of concentration. Table III summarizes the results of this analgesic study. Each dosage shown was the highest attainable for that particular compound in a single intracerebroventricular injection.

Table II—Results of Antagonism Study for 2-Azabicyclo[2.2.2]-octane Analogs of Fentanyl-Type Analgesics

Agonist	Antagonist	Dose of Antagonist, mg/kg	Analgesic Response	Lethality
Morphine[a]	III·HCl	100	6/6	2/8
Morphine	IV·HCl	100	8/8	0/8
Fentanyl[b]	V	100	4/4	0/4
Fentanyl	VI	50	2/2	2/4
Fentanyl	IX	25	4/4	0/4
X[c]	Naloxone	4.0	0/4	0/4

[a] Dose of morphine sulfate = 5.0 mg/kg. [b] Dose of fentanyl citrate = 500 μg/kg. [c] Dose of X = 75 mg/kg.

Table I—Analgesic Activity of the 2-Azabicyclo[2.2.2]octane Analogs of Fentanyl-Type Analgesics and the Positive Controls with 95% Confidence Limits

Compound	AD_{50}	LD_{50}	AD_{50} of Positive Control
III·HCl	Inactive at 100 mg/kg	—[a]	M[b] = 1.32 mg/kg (0.84–2.07)
IV·HCl	Inactive at 100 mg/kg	—[a]	M = 1.32 mg/kg (0.84–2.07)
VII·HCl	60.0 mg/kg (44.1–81.4)	62.5 mg/kg (55.5–70.3)	M = 1.32 mg/kg (0.84–2.07)
VIII·HCl	71.5 mg/kg (61.1–83.7)	91.0 mg/kg (84.9–97.5)	M = 3.0 mg/kg (2.3–4.0)
V	Inactive at 100 mg/kg	—[a]	M = 5.0 mg/kg (1.55–16.0)
VI	Inactive at 100 mg/kg	—[a]	M = 5.0 mg/kg (1.55–16.0) F[c] = 68.5 μg/kg (52.3–89.7)
IX	Inactive at 100 mg/kg	—[a]	M = 5.0 mg/kg (1.55–16.0) F = 68.5 μg/kg (52.3–89.7)
X	43.0 mg/kg (31.2–59.3)	—[d]	M = 5.0 mg/kg (1.55–16.0) F = 68.5 μg/kg (52.3–89.7)

[a] No deaths occurred at 100 mg/kg. [b] M = morphine sulfate. [c] F = fentanyl citrate. [d] Death occurred in two of eight mice at a dose of 150 mg/kg.

Table III—Analgesic Response to the Isomeric *N*-Phenethyl 2-Azabicyclo[2.2.2]octane Analogs of Fentanyl-Type Analgesics in Mice *via* Intracerebroventricular Route with 3% Acacia–Saline as the Vehicle [a]

Compound	Dose, mg/kg	Analgesic Response	
		20 min	60 min
V	3.0	0/4	0/4
VI	5.0	1/4	0/4
IX	20.0	0/4	0/4
X	5.0	1/4	2/4

[a] Morphine sulfate AD_{50} = 0.21 mg/kg; fentanyl citrate AD_{50} = 14.0 μg/kg.

Several conclusions can be drawn from these studies.

1. Since the most potent analog (as the free base) of the eight isomeric compounds prepared had a potency ratio of <1/600 (on the basis of the AD_{50} values determined) when compared to fentanyl citrate, the boat conformations do not appear to contribute to receptor interactions of the fentanyl-type analgesics; this finding suggests the importance of chair conformations in this class of analgesics. A recent report (9) on tropane analogs of fentanyl supports this conclusion.

2. The argument that boat conformations do not contribute to analgesic activity can be applied more readily for V and VI, the two *N*-phenethyl analogs of the 4-substituted piperidine-type analgesics. Analogous structures such as the 2,5-dimethyl derivatives of fentanyl were examined by Riley *et al.* (17) for analgesic activity. Although the exact configurations of the two ring methyl groups were not elucidated, ring methylation at C-2 and C-5 of fentanyl does not seem to destroy the analgesic effect since this compound was still 5% as active as fentanyl. Since V and VI can be regarded as analogs having the C-2 and C-5 methyl groups linked together, with the resultant restriction of the piperidine ring into a boat conformation, the liability of boat conformations was even more obvious.

3. The lack of analgesic activity of III and IV, the two *N*-methyl analogs of the 4-substituted piperidine-type analgesics, is consistent with the report (6) that, unlike the prodine-type analgesics, fentanyl-type analgesics possessing *N*-methyl substituents display weak analgesic activity at best.

4. Since three of the four *N*-methyl and *N*-phenethyl analogs that correspond to 3-substituted piperidine-type analgesics displayed moderate activity and since the *N*-phenethyl analog X was only slightly more active than the corresponding *N*-methyl analogs, positional isomerism apparently accounts for a fundamental alteration in the mode of binding of the molecules at the analgesic receptor.

5. The similar order of analgesic activity observed for the four *N*-phenethyl analogs (V, VI, IX, and X) when the intracerebroventricular route was employed suggests that the differences in apparent analgesic activity truly reflect differences in intrinsic activity and not large differences in disposition. To support this argument, attempts were made to determine the pKa values and partition coefficients of these four analogs. Due to their highly hydrophobic nature and thus their limited solubility, the pKa values that were obtained by following the method of Mathison and Tidwell (18) were questionable, and these determinations were discontinued. A similar fate was encountered in the partition coefficient study. The solubility (in pH 7.4 buffer saturated with octanol) of VI was lower than 2×10^{-6} *M* (or 0.0008 mg/ml). This value was determined by measuring, in duplicate, the UV absorbance (λ_{max} 252.5) prior to and after filtration. The absorbance of the mixture (previously stirred overnight at room temperature) before filtration was invariably far more intense than that after filtration, suggesting that the octanol-saturated buffer was still saturated with VI at that concentration.

6. The abolition of the analgesic effect of X by naloxone and the observation of the Straub tail effect at all doses of X producing analgesia implied the mediation of analgesia through binding to the opiate receptor.

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