Anal. (block dried) ($C_{22}H_{35}N_3O_2 \cdot 2HCl$) C, H, Cl, N.

Similarly prepared were compounds 1, 2, 6, 12, 22, 24, and 25 (Table I).

Acknowledgment. This work was supported by the U.S. Army Medical Research and Development Command under Contract DAMD17-78-C-8001. This is contribution no. 1573 from the Army Research Program on Antiparasitic Drugs. The advice and timely suggestions of Dr. E. A. Steck of the Walter Reed Army Institute of Research are gratefully acknowledged.

Synthesis and Biological Activity of Fluoroalkylamine Derivatives of Narcotic Analgesics

William G. Reifenrath,[1] Edward B. Roche,* Walid A. Al-Turk,

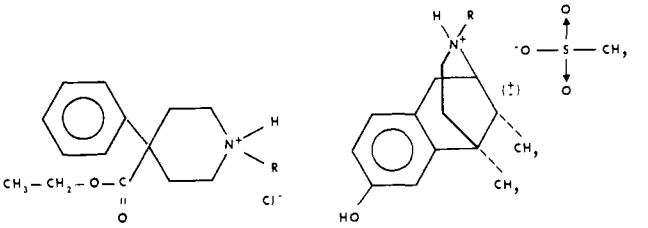
Department of Biomedicinal Chemistry, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 68105

and Howard L. Johnson

Department of Bio-Organic Chemistry, SRI International, Menlo Park, California 94025. *Received February 1, 1980*

N-Ethyl-, *N*-(2-fluoroethyl)-, *N*-(2,2-difluoroethyl)-, and *N*-(2,2,2-trifluoroethyl)-substituted normeperidine (1b–e) and normetazocine (2b–e) derivatives were prepared. The analgesic activities of the compounds were determined in mice. Opiate receptor binding studies, in the presence and absence of sodium ion, were carried out. The antagonist activities of normetazocine derivatives were studied in monkeys. These were further examined in the isolated guinea pig ileum for relative agonist activity. The pK_a values were measured; in vivo agonist activity was lost with weakly basic derivatives. For the normetazocine derivatives, opiate receptor binding data were consistent with guinea pig ileum agonist potency and mouse vas deferens antagonist potency but not with in vivo data. Opiate receptor binding was reduced for the less basic normetazocine derivatives. In the normeperidine series, there was no apparent direct relationship between pK_a and opiate receptor binding. However, a relationship involving the hydrophobic character of the N-substituent is discussed. The *N*-(2-fluoroethyl) derivatives in both series were found to cause convulsions in rats at doses of 40–45 mg/kg ip. Elevated serum citrate levels were found in these rats, implicating in vivo oxidative deamination of the *N*-(fluoroalkyl) substituent to fluoroacetate.

Most narcotic analgesics bear a nitrogen functionality of sufficient basicity to be predominantly protonated at physiological pH.[2] Narcotic analgesic–receptor interactions have, therefore, been conceptualized as involving the protonated amine form of these compounds.[3,4] The positively charged nitrogen atom is thought to bind with a postulated anionic site on receptor macromolecules. Literature reports indicate that reduced basicity results in a loss of narcotic analgesic activity.[5,6] Although these results are consistent with the above hypothesis, it is not known what role steric, distribution, and metabolism factors play in the overall activity of these compounds. Since it is known that β-fluoroalkylamines are significantly weaker bases than their unsubstituted counterparts[7,8] and that the fluorine atom, because of its small van der Waals radius (F = 1.35 Å, H = 1.2 Å),[9] offers minimal steric interference to binding of an analogue at a subcellular macromolecular site, narcotic analgesic derivatives containing this type of substitution are worthwhile candidates to test the importance of nitrogen basicity for activity. In this report, the synthesis and preliminary pharmacological activities of *N*-ethyl-, *N*-(2-fluoroethyl)-, *N*-(2,2-difluoroethyl)-, and *N*-(2,2,2-trifluoroethyl)-substituted 4-phenyl-4-carbethoxypiperidine (normeperidine) (**1b–e**) and (±)-5,9-α-dimethyl-2′-hydroxy-6,7-benzomorphan (normetazocine) (**2b–e**) derivatives are given.



1 2

R
a) H
b) $CH_2 - CH_3$
c) $CH_2 - CH_2F$
d) $CH_2 - CHF_2$
e) $CH_2 - CF_3$

Chemistry. The previously reported *N*-ethylnormeperidine hydrochloride[10] (**1b**) and *N*-ethylnormetazocine hydrobromide[11] (**2b**) derivatives were synthesized for pharmacological comparison purposes. It was found to be more convenient to isolate the latter as the mesylate salt. The *N*-(2-fluoroethyl) derivatives were prepared by reaction of either normeperidine (**1a**) or normetazocine (**2a**) with either 2-fluoroethyl bromide or 2-fluoroethyl tosylate in a nonaqueous solvent. Since preparation of 2-fluoroethyl bromide from 2-bromoethyl tosylate and KF lead to the production of vinyl bromide as a byproduct,[12] the more

(1) Letterman Army Institute of Research, Persidio of San Francisco, CA 94129.
(2) C. G. Farmilo, P. M. Oestreicher, and L. Levi, *Bull. Narc.*, 6, 7 (1954).
(3) A. H. Beckett and A. F. Casy, *J. Pharm. Pharmacol.*, 6, 986 (1954).
(4) D. R. H. Gourley, *Prog. Drug Res.*, 7, 36 (1963).
(5) A. H. Becket and J. V. Greenhill, *J. Med. Pharm. Chem.*, 4, 423 (1961).
(6) R. T. Major and F. Durch, *J. Org. Chem.*, 26, 1867 (1961).
(7) A. L. Henne and J. J. Steward, *J. Am. Chem. Soc.*, 77, 1901 (1955).
(8) R. W. Fuller, H. D. Snoddy, and B. B. Molly, *J. Pharmacol. Exp. Ther.*, 184, 278 (1973).
(9) R. D. Chambers, "Fluorine in Organic Chemistry", Wiley-Interscience, New York, 1973, p 4.
(10) R. H. Thorp and E. Walton, *J. Chem. Soc.*, 559 (1948).
(11) J. H. Ager and E. L. May, *J. Org. Chem.*, 25, 984 (1960).

0022-2623/80/1823-0985$01.00/0

Table I. Opiate Receptor Affinities, Analgesic Activities, and Basicity of *N*-(Fluoroalkyl)-Substituted Normeperidine (1) and Normetazocine (2) Derivatives

compd	N-substituent	opiate receptor binding:[a] IC_{50}, nM −NaCl	opiate receptor binding:[a] IC_{50}, nM +NaCl	sodium response ratio[b]	analgesia:[c] ED_{50}, mg/kg (90% CL)	pK_a (±SD)	% N-protonate at pH 7.4
1b	CH_3CH_2-	5000	120000	24.0	4.4 (3.2-6.1)	9.03 (0.06)	98
1c	FCH_2CH_2-	20000	64000	3.2	19.4 (12.7-29.8)	7.43 (0.04)	50
1d	F_2CHCH_2-	85000	110000	1.3	inact to 100	5.23 (0.08)	1
1e	F_3CCH_2-	10000	60000	6.0	inact to 100	3.13 (0.04)	0
2b	CH_3CH_2-	180	200	1.1	44.7 (17.8-72.1)	9.42 (0.03)	99
2c	FCH_2CH_2-	50	100	2.0	9.1 (6.4-12.8)	8.35 (0.05)	89
2d	F_2CHCH_2-	1000	1150	1.2	9.7 (6.3-14.8)	6.52 (0.05)	12
2e	F_3CCH_2-	1700	2500	1.5	inact/toxic at 40	4.56 (0.08)	0
morphine		10	250	25.0[d]	1.2		
meperidine		500	40000	80[e]			

[a] Inhibition of opiate receptor binding, expressed as the concentration of drug which reduced specific [^{3}H]naloxone binding by 50%. [b] The ratio of IC_{50} values for incubations in the presence of 100 mM NaCl to those incubations in its absence. [c] Mouse hot-plate test[13] data, courtesy of Dr. E. L. May (NIH). Compounds administered subcutaneously in normal saline, except for 1e which was given in 40% propylene glycol. [d] Data from ref 34. [e] Data from ref 35.

Table II. Agonist and Antagonist Potencies in the Guinea Pig Ileum and Mouse Vas Deferens[a]

compd	guinea pig ileum: ID_{50},[b] nM	guinea pig ileum: agonist potency (normorphine = 1)	mouse vas deferens: K_e,[c] nM	mouse vas deferens: antagonist potency (naloxone = 1)
2c	50.8 ± 9.2 (4)	1.29 ± 0.22 (5)	558 ± 109 (4)	0.007
2d	453 ± 83 (4)	0.16 ± 0.02 (4)	2930 ± 720 (4)	0.001
2e	infinite	0		

[a] The values are the means ± SE; test data are courtesy of Drs. E. L. May (NIH) and H. W. Kosterlitz (University of Aberdeen, Scotland). [b] Concentration necessary to reduce electrically stimulated contractions by 50%. [c] Equilibrium dissociation constant. Concentration required to produce a 50% reduction in the response produced by normorphine.

facile and cleaner preparation of the tosylate of 2-fluoroethanol was used in later experiments. 2,2-Difluoroethyl trifluoromethanesulfonate or 2,2,2-trifluoroethyl trifluoromethanesulfonate was reacted with the appropriate secondary amine to form the *N*-(2,2-difluoroethyl) and *N*-(2,2,2-trifluoroethyl) derivatives, respectively.

Biological Activity Data. Analgesic activity, as determined in the mouse hot-plate test,[13] is listed in Table I.

Opiate receptor binding was measured by the method of Pert and Snyder.[14] The opiate receptor affinities and sodium response ratios are given in Table I, together with pK_a values for derivatives **1b–e** and **2b–e**. Using the Henderson–Hasselbach equation,[15] the percentage of the compound that would be N protonated at pH 7.4 was estimated.

Results

Fluorine substitution in the N-substituent decreased in vivo analgesic activity in the normeperidine series but increased such activity in the normetazocine series (Table I). The IC_{50} values for stereospecific binding increased as the pK_a (percent N protonation) decreased in the normetazocine series, but this did not parallel in vivo activity (Table I). In the normeperidine series, there was no apparent relationship between pK_a and stereospecific binding (Table I).

In the guinea pig ileum assay[16] (Table II), *N*-(2-fluoroethyl)normetazocine (**2c**) was about as active as normorphine, which is equipotent with morphine in this assay.[17] This is a greater activity than would be expected from the in vivo results shown for mice (Table I). Agonist activity (Table II) was reduced by further fluorine substitution in agreement with in vivo data. None of the fluoroalkylnormetazocines exhibited antagonist activity in the guinea pig ileum.

The mouse vas deferens preparation allows measurement of antagonist activity of compounds with high agonist/antagonist ratios.[18] Using this assay, compounds **2c** and **2d** were found to have weak antagonist activity, 0.7 and 0.1% of naloxone, respectively; compound **2e** was inactive (Table II).

The IC_{50} sodium response ratios derived from the stereospecific binding assays suggest that fluorine substitution in the N-substituent should enhance antagonist activity in the normeperidine series (Table I). This aspect has not been evaluated in vivo or in vitro for this series. However, in general, opiates exhibiting a sodium response ratio above 12.0 have been predominantly agonists, while antagonists and mixed agonist/antagonists exhibit much lower ratios.[19] *N*-Ethylnormetazocine (**2b**) exhibited a low ratio, and this was not substantially altered by fluorine substitution (Table I). Of the three normetazocine derivatives tested in the guinea pig ileum and mouse vas deferens preparations (Table II), the one having both the highest agonist

(12) W. F. Edgell and L. Parts, *J. Am. Chem. Soc.*, 77, 4899 (1955).
(13) N. B. Eddy and D. Leimbach, *J. Pharmacol. Exp. Ther.*, 107, 385 (1953).
(14) C. B. Pert and S. H. Snyder, *Mol. Pharmacol.*, 10, 868 (1974).
(15) A. N. Martin, J. Swarbrick, and A. Cammarita, "Physical Pharmacy", 2nd ed., Lea and Febiger, Philadelphia, Pa., 1969, p 237.
(16) H. W. Kosterlitz and A. J. Watt, *Br. J. Pharmacol.*, 33, 266 (1968).
(17) H. W. Kosterlitz, J. A. H. Lord, and A. J. Watt, in "Agonist and Antagonist Actions of Narcotic Analgesic Drugs"; H. W. Kosterlitz, H. O. J. Collier, and J. E. Villarreal, Eds., University Park Press, London, 1973, p 50.
(18) J. Hughes, H. W. Kosterlitz, and F. M. Leslie, *Br. J. Pharmacol.*, 51, 139 (1974).
(19) C. B. Pert and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, 70, 2242 (1973), *Mol. Pharmacol.*, 10, 868 (1974); C. B. Pert, S. H. Snyder, and E. L. May, *J. Pharmacol. Exp. Ther.*, 196, 316 (1976); C. B. Pert, S. H. Snyder, and P. S. Portoghese, *J. Med. Chem.*, 19, 1248 (1976); R. S. Wilson and M. E. Rogers, *ibid.*, 18, 240 (1975).

Table III. Serum Citrate Levels in the Rat

compd	dose, mg/kg ip (no. of animals)	serum citrate level, mg/mL (SE)
1c	40 (6)	81.0 (5.0)
2c	45 (7)	121.0 (3.2)
sodium fluoroacetate	12.5 (5)	132.0 (3.3)
saline	(5)	41.0 (2.0)

potency and the highest antagonist potency (**2c**) also had the highest affinity for stereospecific receptor binding (Table I).

The low activity of **2e** may be ascribed to its weak basicity and virtual absence of protonation at physiological pH in spite of a reasonable degree of receptor affinity. A similar conclusion can be drawn with regard to the fluorinated normeperidines (**1d** and **1e**). Although receptor affinity (IC_{50}) appears to be related to protonation in the case of the normetazocines, this does not appear to be entirely true for the normeperidines. Furthermore, protonation appears to influence analgesic potency apart from effects on receptor affinity.

Compounds **2c–e** were examined in morphine-dependent monkeys;[20,21] compound **2c**, at a dose of 0.5 mg/kg sc, precipitated withdrawal in nonwithdrawn morphine-dependent monkeys. Higher doses of **2c** (up to 4 mg/kg sc) precipitated a more severe withdrawal. In nonwithdrawn morphine-dependent monkeys, derivative **2d** (5 mg/kg sc) did not precipitate abstinence signs. Compound **2d**, at doses of 5, 10, and 20 mg/kg sc, and compound **2e**, at doses of 3, 6, and 12 mg/kg sc, had no effect on suppressing abstinence signs in withdrawn morphine-dependent monkeys. Compound **2e** showed little or no activity in the mouse tail-flick antagonist vs. morphine test;[22] further testing in monkeys was, therefore, not warranted. With the possible exception of **2d**, these results are consistent with in vitro data (Tables I and II) and confirm the antagonist activity of **2c**.

It was expected that compounds **1c** and **2c** would exhibit toxic reactions in animals as a result of oxidative N-dealkylation of the 2-fluoroethyl substituent to fluoroacetate. Characteristic clonic and tonic convulsions have been reported in rats poisoned with fluoroacetate.[23] A dose of sodium fluoroacetate (12.5 mg/kg ip) was selected which produced convulsions in all of the rats in the experimental group. Equimolar doses of **1c** and **2c** also produced similar convulsive behavior in all of the rats. As seen in Table III, all three of these compounds caused significantly higher serum citrate levels than the controls (saline). Since elevated citrate levels have been found in association with fluoroacetate poisoning,[24] these results implicate oxidative dealkylation of the *N*-(2-fluoroethyl) substituent in **1c** and **2c** to fluoroacetate. Compounds **1c** and **2c**, at analgesic doses (Table I), did not produce convulsions in mice and monkeys. Although these doses were somewhat lower than those used in the citrate determinations (Table III), **1c** was found to produce convulsions in rats in doses as low as 10 mg/kg ip. It has been reported that mice are more resistant to the toxic effects of fluoroacetate and that, in rats, sodium fluoroacetate has an LD_{50} of 15 mg/kg ip.[24] As seen in Table IV, rats were more sensitive than mice to the convulsive and lethal effects of both sodium fluoroacetate and compound **1c**. Although these results suggest that metabolism-induced fluoroacetate plays a role in the toxic manifestations of **1c** and **2c**, other factors (e.g., a convulsant property of the intact molecule) cannot be excluded.

Discussion

Analgesic activities (agonist and antagonist) in vivo are dependent upon receptor affinities, ligand protonation, metabolism, transport, and distribution. Protonation can affect transport and, at least in the case of the normetazocines, receptor affinity. If protonation is necessary for intrinsic (agonist) activity, one would expect to see increased antagonist activity with decreased basicity when receptor binding remains relatively constant. Such a trend can be seen in the sodium response ratios for the normeperidine derivatives (**1b–e**) (Table I). The apparent lack of such a trend in the normetazocine derivatives (**2b–e**) (Table I) may well be due to the decrease in both receptor affinity and basicity resulting in a relatively constant sodium response ratio. These results may reflect a difference in binding mode between the two structural types as suggested previously by Portoghese.[25] The loss of both agonist and antagonist activities in vivo for compounds which are virtually resistant to protonation at physiologic pH (**1e** and **2e**) could be due to inefficient transport to receptor sites. This seems plausible in view of the reasonable relative in vitro receptor affinities of compounds **1e** and **2e**.

In seeking a relationship between structure, properties, receptor binding affinities, other in vitro data, and in vivo analgesic potency, the influence of the fluorine substitution on both the lipophilic properties (log *P*) and pK_a must be considered. From fragment constant analysis,[26] the influence of successive fluorine substitution on the number 2 carbon of the *N*-ethyl group can be quantitatively approached. The methyl portion of the *N*-ethyl group (no. 2 carbon in **1b** and **2b**) contributes 0.89 log *P* (f_{CH_3}) unit to the lipophilic character of the respective molecules. The substitution of one fluorine in place of a hydrogen on the methyl group (**1c** and **2c**) reduces the lipophilic contribution by 0.61 log *P* unit ($f_{CH_2F} = 0.28$). Substitution of a second fluorine for hydrogen (**1d** and **2d**) has a minimal effect on lipophilicity ($f_{CHF_2} = 0.27$) due to shielding of the polar contribution by the geminal halogens.[34] A third fluorine substitution (**1e** and **2e**) introduces a stronger geminal effect,[26] leading to an increase in the lipophilic contribution ($f_{CF_3} = 0.65$), but still less than that of a methyl group. Hence, fluorine substitution on the aliphatic N-substituent in both series of analgesics results in an overall decrease in lipophilic character, but the decrease is not a linear function of the number of geminal fluorine substitutions. In fact, the trifluoromethyl group is approximately halfway between the methyl group and the mono- or difluoromethyl group in positive contribution to log *P*.

As noted from pK_a values and calculated percent ionizations in Table I, successive fluorine substitution causes a dramatic decrease in basicity and, hence, percent of protonated amine at pH 7.4 in both series of compounds. Differences between *N*-ethyl and *N*-(2,2,2-trifluoroethyl)

(20) H. H. Swain and M. H. Seevers, "Committee on Problems of Drug Dependence", 768 (1976).
(21) M. D. Aceto, L. S. Harris, W. L. Dewey and R. L. Balster, *Probl. Drug Depend.*, 788 (1976).
(22) W. L. Dewey and L. S. Harris, *J. Pharmacol. Exp. Ther.*, 179, 652 (1971).
(23) R. A. Peters, *Proc. R. Soc. London, Ser. B*, 139, 143 (1952).
(24) F. L. M. Pattison, *Nature (London)*, 172, 1139 (1953).
(25) P. S. Portoghese, *J. Med. Chem.*, 8, 609 (1965).
(26) C. Hansch and A. Leo in "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley-Interscience, New York, 1979, pp 18–43.

Table IV. Species Variations to the Toxic Effects of Sodium Fluoroacetate and N-(2-Fluoroethyl)normeperidine (1c)

compd	dose, mg/kg, ip	no. of animals	species	no. of animals convulsing	deaths
sodium fluoroacetate	10.0	6	rat	5/6	6/6
	10.0	9	mouse	0/9	2/9
1c	20.0	6	rat	6/6	3/6
	20.0	6	mouse	0/6	0/6
	40.0	6	mouse	0.6	0/6
	60.0	6	mouse	3/6	3/6

substituents (**1b** vs. **1e** and **2b** vs. **2e**) are on the order of 5–6 pK_a units, and the latter derivatives are not protonated at physiological pH. The significance of this fact may well be different with respect to transport and receptor binding. The more weakly basic compounds may have improved lipid solubility and, hence, improved transport properties. On the other hand, the absence of significant numbers of positively charged molecules may be detrimental to ligand binding to receptors. It is interesting to note that there may be a balance between increased lipophilicity, due to decreased basicity, and decreased lipophilicity, due to increased fluorine substitution.

Considering the effect of the above-mentioned properties on the observed changes in in vitro opiate receptor IC_{50} values (Table I), it would appear that both the hydrophobic character of the N-alkyl side chain and the extent of protonation of the amine play a role in ligand binding to the receptor in the normeperidine series (**1b–e**). The decrease in binding in the absence of sodium for **1b** through **1d** follows the decrease in the extent of protonation of the amine. In addition, the N-alkyl substituent of **1c** and **1d** is less hydrophobic than the N-ethyl group (**1b**). In as much as the hydrophobic character of this substituent contributes to ligand binding, this reduction in hydrophobicity may be significant. The increase in binding of the 2,2,2-trifluoroethyl derivative (**1e**) is unexpected in view of the absence of protonated molecules at pH 7.4. However, the hydrophobic character of this substituent is more than twice that of **1c** and **1d** and lies between those and the nonfluorinated derivative (**1b**). All other factors being equal, this seems to reflect a major hydrophobic contribution of this substituent to ligand binding.

It is interesting to note that both compounds **1d** and **1e** were inactive in in vivo analgesia assays (Table I) in spite of the relatively strong in vitro binding of **1e**. This inactivity may reflect poor transport into the CNS and/or the necessity of the protonated form for intrinsic activity at the receptor site. If one compares the relative lipophilic character of the N-substituents and the relative percentages of un-ionized (more lipophilic) species between compound **1e** and the active compound **1c**, it becomes difficult to accept poor transport properties as a major contribution to the absence of activity of **1e**. The availability of positively charged forms in analgesic receptor interactions of these normeperidine derivatives may well be important to intrinsic activity. Additionally, the rate at which these compounds are metabolized is another unknown factor having a major influence on their activity.

With regard to the normetazocine series (**2b–e**), the greatest influence on ligand binding in the in vitro opiate receptor assay appears to be exerted through the effect of successive fluorine substitution on the pK_a of the protonated amine group (Table I). The electron-withdrawing effect of the fluorines on the basicity of the amine is not as dramatic in this series as it was in the normeperidine series. A comparison of compounds **2b** and **2c** reveals a difference in binding potency that is relatively insignificant when considered over the range of values for this series. Similarly, the difference in percentage of amine protonated between these two is only 10%. The decrease in hydrophobic character for **2c** does not seem to cause any decrement in ligand binding. A second fluorine substitution (**2d**) does not significantly alter the hydrophobic properties of the N-alkyl group (vide supra), but it does produce a sizable drop in the pK_a and the percentage of protonated amine, as well as a 20-fold decrease in ligand binding. The trifluoroethyl group of **2e** reduces the pK_a to the extent that ionized forms are virtually absent at pH 7.4, and ligand binding decreased another 70%. The hydrophobic influence of this group is twice that of the corresponding groups on **2c** and **2d** but, unlike the normeperidine series, does not seem to have a positive influence on receptor binding. The overall ligand binding to receptors in this series of benzomorphan derivatives does not appear to be influenced by hydrophobic groups of this size on the nitrogen but does appear to be influenced by the availability of positively charged species. The apparent differences between the 4-phenylpiperidines (series 1) and the benzomorphans (series 2) with regard to properties influencing binding are not unexpected and may be due to differences in binding modes[25] as alluded to previously.

An unexpected observation concerns the in vivo analgesic potency of compounds **2c** and **2d** (Table I). Although the receptor affinity of **2d** was markedly less than **2c**, the analgesic potencies were not significantly different. It may well be that overall transport processes are responsible for this effect. With the exception of ionization, **2c** and **2d** should have similar lipophilic properties. The decreased ionization of **2d** may facilitate its transport into the CNS to the extent that its concentration at receptor sites is higher than **2c**, leading to similar amounts of ionized species to activate analgesic receptors. If this is indeed the case, a greater potency difference would be expected in in vitro assays such as guinea pig ileum and mouse vas deferens. The data in Table II show a sizeable reduction in agonist potency from **2c** to **2d** in the guinea pig ileum assay. The antagonist potency in the mouse vas deferens is consistent, showing a reduction from **2c** to **2d** (Table II). These data would tend to support the hypothesis that in vivo transport differences account for the similarities in analgesic potency between these two compounds. Studies based on this hypothesis utilizing labeled derivatives and including all members of both series are beginning. It should be noted that in vivo and in vitro activity was lost for compound **2e** in which basicity was reduced to such an extent that protonation in physiological pH was negligible.

The metabolism and mechanism of the toxicity of the N-fluoroethyl derivatives **1c** and **2c** mentioned under Results are currently under investigation.

Experimental Section

Melting points were taken on a Mel-Temp capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were recorded at 60 MHz on a Varian A-60 or EM-360 spectrometer. Infrared spectra were obtained using a Beckman IR-18A spectrophotometer. Infrared and NMR measurements were determined for all reported compounds and were consistent with the assigned structures. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Where analyses are reported by symbols of the elements, analytical results were within 0.4% of the calculated value.

The pK_a values of compounds 1b–e and 2b–e were obtained by potentiometric titration in distilled water at 24 °C. A sufficient amount of compound was dissolved in 20 mL of water to give a concentration of 5 to 7.5 × 10^{-3} M. The titrant (0.1039 M NaOH) was added with a microburet (Model 1MB 1300, Manostat Corp., N.Y.) and the pH change followed with a Beckman Zeromatic Model SS-3 pH meter. Each compound was run in triplicate.

Animals used in toxicity studies were obtained from Sasco, Omaha, Neb. Male Swiss Webster mice weighing 20–25 g and male Sprague–Dawley rats weighing 200–225 g were employed. Compounds 1c, 2c, and sodium fluoroacetate were dissolved in normal saline in a concentration to allow an injection volume of 0.2 mL and were administered ip. Animals were individually housed and were observed for a period of 3 h after injection. The number of animals experiencing either clonic or tonic convulsions during this time interval was noted, as well as the number of animals dead 24 h postinjection.

Serum citrate levels were measured in rats by the procedure of Camp and Farmer.[27] The rats were decapitated, and blood samples were collected in 50-mL centrifuge tubes and centrifuged on a Sorvall Type SS-1 centrifuge to obtain the serum, for those substances producing convulsions. The animals were sacrificed at the onset of convulsions. Control rats given only normal saline were sacrificed at comparable time intervals.

Normeperidine (1a),[28] normetazocine (2a),[29] *N*-ethylnormeperidine (1b),[10] trifluoromethanesulfonic anhydride,[30] and 2,2,2-trifluoroethyl trifluoromethanesulfonate[31] were synthesized by published procedures.

2-Fluoroethyl Tosylate. This compound was prepared from 2-fluoroethanol and *p*-toluenesulfonyl chloride using standard laboratory procedures:[32] 81% yield of product was obtained; bp 140 °C (0.10 mm).

2,2-Difluoroethanol. A dry 250-mL three-neck flask containing 50 mL of dry ethyl ether and equipped with a nitrogen inlet valve, pressure equalizing funnel, reflux condenser topped with a valve leading to a bubbler (mineral oil), and a magnetic stirrer was flushed throughly with nitrogen. Using a syringe, the flask was then charged with 11.5 mL (114 mmol) of borane–methyl sulfide complex. Difluoroacetic acid (10.0 g, 104 mmol) dissolved in 10 mL of dry ethyl ether was placed in the addition funnel. The difluoroacetic acid was then added with stirring over a period of 2 h. The reaction mixture was stirred an additional 8 h at room temperature. Then, 10 mL of H_2O was cautiously added to decompose the reaction mixture. The reaction mixture was then filtered, and the precipitate was washed with ether. The ether filtrates were combined and dried over anhydrous sodium sulfate. The ether solution was then distilled at atmospheric pressure over drierite, and a fraction boiling at 92–94 °C was collected to give 4.6 g (56% yield) of difluoroethanol: lit.[33] bp 95.5–96 °C (1 atm).

2,2-Difluoroethyl Trifluoromethanesulfonate. This compound was prepared using a procedure similar to the preparation of 2,2,2-trifluoroethyl trifluoromethanesulfonate.[32] In a two-neck 50-mL flask equipped with a reflux condenser was placed 2.75 g (34 mmol) of difluoroethanol and 9.25 g (34 mmol) trifluoromethanesulfonic anhydride. The reaction mixture was refluxed for 1 h. After cooling, the reaction mixture was poured into 25 mL of 5% aqueous sodium bicarbonate and extracted immediately with an equal volume of ethyl ether. The ether layer was separated and dried over anhydrous sodium sulfate. The filtered ether extract was then distilled, and the fraction boiling at 118 °C, 1 atm, was collected, giving 4.1 g (57% yield) of product.

***N*-(2-Fluoroethyl)normeperidine Hydrochloride (1c).** In a 25-mL flask, equipped with a condenser with the open end connected to a mineral oil bubbler, were placed 1.65 g (13 mmol) of 2-fluoroethyl bromide, 2.6 g (26 mmol) of triethylamine, and 10 mL of acetone. In a nitrogen atmosphere (normeperidine free base readily forms a carbonate salt with CO_2 in the atmosphere[29]), 3.0 g (13 mmol) of normeperidine was added to the flask. The reaction mixture was stirred and refluxed for 2 h, and at the end of this time the solvent was removed under reduced pressure. Ethyl ether was added to the residue, and the insoluble material filtered from the ether solution. Carbon dioxide was then bubbled into the ether solution to precipitate any unreacted normeperidine as the carbonate salt. The ether solution was then filtered and the ether was removed under reduced pressure to leave an oil, which was distilled at 140 °C, 0.2 mm pressure, to give 2.9 (80% yield) of product. Hydrochloride salt (acetone–ethyl ether): mp 164–166 °C. Anal. ($C_{16}H_{23}ClFNO_2$) C, H, N, F.

***N*-(2,2-Difluoroethyl)normeperidine Hydrochloride (1d).** In a 25-mL flask equipped as for 1c were placed 1.4 g (6.5 mmol) of 2,2-difluoroethyl trifluoromethanesulfonate and 15 mL of acetone. In a nitrogen atmosphere, 3.0 g (13 mmol) of normeperidine was added to the flask. The reaction mixture was stirred, refluxed for 5 h, and worked up as for 1c: 1.6 g (80% yield based on the triflate) of product was obtained; bp 140 °C (0.25 mm). Hydrochloride salt (acetone–ethyl ether): mp 196–198 °C. Anal. ($C_{16}H_{22}ClFNO_2$) C, H, N, F.

***N*-(2,2,2-Trifluoroethyl)normeperidine Hydrochloride (1e).** In a 25-mL flask equipped as for 1c were placed 2.0 g (8.6 mmol) of 2,2,2-trifluoroethyl trifluoromethanesulfonate and 15 mL of acetone. In a nitrogen atmosphere 4.0 g (17 mmol) of normeperidine was added to the flask. The reaction mixture was stirred and refluxed for 5 h and was then worked up as for 1c: 2.2 g (81% yield based on the triflate) of product was obtained: bp 120–125 °C (0.10 mm). Hydrochloride salt (acetone): mp 174–176 °C. Anal. ($C_{16}H_{23}ClF_3NO_2$) C, H, F, N.

***N*-Ethylnormetazocine Methanesulfonate (2b).** In a 25-mL flask equipped with a reflux condenser and magnetic stirrer were placed 1.00 g (4.61 mmol) of normetazocine (2a), 0.72 g (4.61 mmol) of ethyl iodide, 0.76 g (9.0 mmol) of sodium bicarbonate, and 15 mL of ethanol. The flask was stirred and heated in an oil bath for 8 h at 65 °C. At the end of this time, the reaction mixture was allowed to cool, and the solvent was evaporated in vacuo. Equal volumes (50 mL) of 10% hydrochloric acid and ether were then added to the residue, and the acid layer was separated and neutralized with concentrated aqueous ammonia. The resultant precipitate was extracted into ether, and the ether solution was washed with water and dried over anhydrous sodium sulfate. To the filtered ether solution was added 0.44 g (4.6 mmol) of methanesulfonic acid dissolved in a small amount of ether. The resulting precipitate was collected by filtration and dissolved in equal volumes (2.5 mL) of methanol and acetone. Anhydrous ether was added to the cloud point, and the solution was refrigerated overnight to effect crystallization. The crystals that formed were filtered from the mother liquor and dried. In this manner, 0.91 g (58% yield) of product was obtained as the mesylate salt: mp 207–208 °C. Anal. ($C_{17}H_{27}NO_4S$) C, H, N. This compound has been previously prepared as the HBr salt.[11]

***N*-(2-Fluoroethyl)normetazocine Methanesulfonate (2c).** In a 25-mL flask equipped with a reflux condenser and magnetic stirrer were placed 1.00 g (4.6 mmol) of normetazocine (2a), 1.00 g (4.6 mmol) of 2-fluoroethyl tosylate, 0.76 g (9.0 mmol) of sodium bicarbonate, and 15 mL of dry dimethylformamide. The reaction mixture was stirred and heated in an oil bath for 8 h at 80 °C. The reaction mixture was worked up as for 2b to yield 0.86 g (52% yield) of product as the mesylate salt: mp 201–203 °C. Anal. ($C_{17}H_{26}FNO_4S$) C, H, F, N. [Attempts to prepare this compound in hydroxylic solvents yielded no product containing the *N*-(2-fluoroethyl) group.]

***N*-(2,2-Difluoroethyl)normetazocine Methanesulfonate (2d).** In a 25-mL flask equipped with a reflux condenser and magnetic stirrer were placed 1.00 g (4.6 mmol) of normetazocine,

(27) B. J. Camp and L. Farmer, *Clin. Chem.*, 13, 501 (1967).
(28) O. Eisleb, *Ber. Dtsch. Chem. Ges.*, 74, 1433 (1941).
(29) T. Kametani, K. Kigasawa, M. Hiiragi, T. Hayassaka, N. Wagatsuma, and K. Wakisake, *J. Heterocycl. Chem.*, 6, 43 (1969).
(30) J. Burdon, I. Farazmand, M. Stacy, and J. C. Tatlow, *J. Chem. Soc.*, 2574 (1957).
(31) J. Burdon and V. C. R. McLaughlin, *Tetrahedron*, 21, 1 (1965).
(32) J. H. Brewster and C. J. Ciotti, Jr., *J. Am. Chem. Soc.*, 77, 6214 (1955).
(33) F. Swarts, *Chem. Zentralbl.*, 74, 436 (1903).
(34) J. I. DeGraw, J. A. Lawson, J. L. Crase, H. L. Johnson, M. Ellis, E. T. Uyeno, G. H. Loew, and D. S. Berkowitz, *J. Med. Chem.*, 21, 415 (1978).
(35) C. B. Pert, S. H. Snyder, and P. S. Portoghese, *J. Med. Chem.*, 19, 1248 (1976).

0.985 g (4.6 mmol) of 2,2-difluoroethyl trifluoromethanesulfonate, 0.76 g (9.0 mmol) of sodium bicarbonate, and 15 mL of ethanol. The reaction mixture was stirred and refluxed for 8 h. The reaction mixture was worked up as for **2b**, except that the mesylate salt of the product was crystallized from acetone and ether. In this manner, 1.07 g (62% yield) of product was obtained: mp 266–268 °C. Anal. ($C_{17}H_{25}F_2NO_4S$) C, H, F, N.

***N*-(2,2,2-Trifluoroethyl)normetazocine Methanesulfonate (2e).** In a 10-mL flask equipped with a reflux condenser and magnetic stirrer were placed 0.375 g (1.73 mmol) of normetazocine (**2a**), 0.40 g (1.73 mmol) of 2,2,2-trifluoroethyl trifluoromethanesulfonate, 0.29 g (3.45 mmol) of sodium bicarbonate, and 5 mL of ethanol. The reaction mixture was stirred and refluxed for 8 h and worked up as for **2b**, except that the mesylate salt of the product was crystallized from acetone. In this manner, 0.44 g (64% yield) of product was obtained: mp 276 °C (dec). Anal. ($C_{17}H_{24}F_3NO_4S$) C, H, F, N.

Acknowledgment. The authors are indebted to Dr. E. L. May, National Institutes of Health, Bethesda, Md., for the mouse hot-plate tests; Dr. H. W. Kosterlitz, University of Aberdeen, Scotland, for guinea pig ileum and mouse vas deferens assays; Mrs. Marie Ellis, SRI International, for opiate receptor binding assays; Drs. H. H. Swain and M. H. Seevers, Department of Pharmacology, The University of Michigan, and Drs. M. D. Aceta, L. S. Harris, W. L. Dewey, and R. L. Balster, Department of Pharmacology, Virginia Commonwealth University, for screening compound **2c–e** in monkeys; and Judy Williams for technical assistance. This research was supported in part by an NSF Traineeship.

Demethyl Analogues of Psychoactive Methoxyphenalkylamines: Synthesis and Serotonin Receptor Affinities

Richard A. Glennon,* Stephen M. Liebowitz, Dianne Leming-Doot,

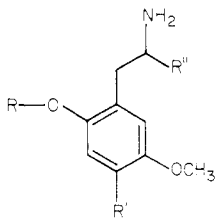
Department of Pharmaceutical Chemistry, School of Pharmacy

and John A. Rosecrans

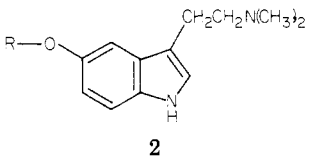
Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.
Received March 24, 1980

Mono-*O*-demethylation of several 2,5-dimethoxyphenalkylamines increases their affinity for the serotonin receptors of the isolated rat fundus preparation. In several instances, demethylation of methoxyphenalkylamines results in compounds which produce an antagonism which is not of a competitive nature. With respect to 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), demethylation of the 2-methoxy group alters affinity in a manner which parallels that observed upon demethylation of 5-methoxy-*N*,*N*-dimethyltryptamine. Using a discriminative stimulus paradigm, behavioral studies with rats reveal that the 2-hydroxy analogue, but not the 5-hydroxy analogue, of DOM produces effects (interoceptive cues) similar to those produced by 5-methoxy-*N*,*N*-dimethyltryptamine.

We have previously reported that certain substituted phenalkylamines (i.e., phenethylamine and phenylisopropylamine derivatives) possess an affinity for the serotonin (5-hydroxytryptamine, 5-HT) receptors of the isolated rat fundus preparation.[1,2] 2,5-Dimethoxy-substituted derivatives (e.g., **1**, R = Me) possess the highest affinities; 4-methylation (e.g., **1**, R = R′ = Me) further enhances affinity.



1



2

A number of attempts have been made to relate the structures of the psychoactive phenalkylamines to the structures of indolealkylamines by varying the orientation of the aromatic moiety and/or the conformation of the side chain.[3-5,8] Kang and Green have noted that the structure of LSD possesses both phenalkylamine and indolealkylamine molecular subfragments;[5] hence, this classical model requires the least manipulation, with respect to reorientation of the aromatic or side-chain functionalities, in order to visualize structural similarities. Though no presupposition is being made as to which is the most valid model, the classical model was used to generate a working hypothesis. 5-Methoxy-*N*,*N*-dimethyltryptamine (5-OMe-DMT; **2**, R = Me) possesses a rather high affinity for the 5-HT receptors of the rat fundus preparation. O-Demethylation of the methoxy group of 5-OMe-DMT to give bufotenine (**2**, R = H) results in a twofold increase in affinity (i.e., pA_2 values are 7.08 and 7.41, respectively). According to the classical model, the 5 position of the tryptamine analogues would correspond to the 2 position of the phenalkylamines. Thus, it was of interest to determine if the demethylation of 2-methoxyphenalkylamines would have an effect on affinity comparable to that seen upon demethylation of 5-OMe-DMT.

Chemistry. Synthesis of desired 2-hydroxy analogues **5d–f** began with the commercially available 2-hydroxy-5-

(1) Glennon, R. A.; Liebowitz, S. M.; Mack, E. C. *J. Med. Chem.* **1978**, *21*, 822.
(2) Glennon, R. A.; Liebowitz, S. M.; Anderson, G. M. *J. Med. Chem.* **1980**, *23*, 294.
(3) Snyder, S. H.; Richelson, E. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *60*, 206. (b) Clothia, C.; Pauling, P. *Ibid.* **1969**, *63*, 1063. (c) Baker, R. W.; Clothia, C.; Pauling, P. *Mol. Pharmacol.* **1973**, *9*, 23. (d) Nichols, D. E.; Pfister, W. R.; Yim, G. K. W.; Cosgrove, R. J. *Brain Res. Bull.* **1977**, *2*, 169. (e) Nichols, D. E.; Pfister, W. R.; Yim, G. K. W. *Life Sci.* **1978**, *22*, 2165.
(4) See discussions in *Neurosci. Res. Prog. Bull.* **1970**, *8*, 1–152.
(5) Kang, S.; Green, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 62.
(6) Dean, F. M.; Goodchild, J.; Houghton, L. E.; Martin, J. A.; Morton, R. B.; Parton, B.; Price, A. W.; Somvichien, N. *Tetrahedron Lett.* **1966**, 4153.
(7) Kier, L. B.; Glennon, R. A. in "Quantitative Structure Activity Relationships of Analgesics, Narcotic Antagonists and Hallucinogens"; Barnett, G.; Trsic, M.; and Willette, R. E, Eds.; U.S. Government Printing Office: Washington D.C., 1978; p 159.
(8) Zweig, J.; Castagnoli, N. *J. Med. Chem.* **1977**, *20*, 414.

0022-2623/80/1823-0990$01.00/0