Roles of Two Basic Nitrogen Atoms in 1-Substituted 4-(1,2-Diphenylethyl)piperazine Derivatives in Production of Opioid Agonist and Antagonist Activities

Kagayaki NATSUKA,* Yoshinori NISHIKAWA, and Hideo NAKAMURA[1)]

Discovery Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33–94, Suita, Osaka 564–0053, Japan.
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To ascertain roles of the two basic nitrogen atoms in 1-substituted 4-[2-(3-hydroxyphenyl)-1-phenylethyl]-piperazine derivatives (1) in the expression of opioid agonist and antagonist activities, a methine group (CH) was isosterically substituted for nitrogen atom at the 1-position (*N*-1) in compound 1 to obtain 4-substituted 1-[2-(3-hydroxyphenyl)-1-phenylethyl]piperidine derivatives (2). Their analgesic action and ability to produce physical dependence (jump-producing activity) as the μ-opioid receptor specific *in vivo* actions, and narcotic antagonist action in mice were compared with those of compound 1. Results of this study showed that, in cases of the racemate and the (*S*)-(+) enantiomer, opioid agonist activities (analgesic and jump-producing activities) were not greatly affected by the methine-substitution for *N*-1 in compound 1, but that the narcotic antagonist activity of the (*R*)-(−) enatiomer was abolished by this substitution. It thus appears that *N*-1 in compound 1 contributes to the expression of narcotic antagonist activity, whereas the nitrogen atom at the 4-position corresponds to the tyramine moiety necessary for the expression of μ-opioid agonist activity.

Key words μ-opioid agonist; opioid antagonist; diphenylethylpiperazine; role of two basic nitrogen atoms

Morphine is known to produce its specific pharmacological activities of analgesic action and physical dependence liability through the μ-opioid receptor binding.[2,3)] A morphine molecule has a 2-(4-hydroxyphenyl)ethylamine (tyramine) moiety possibly essential for opioid receptor binding.[3,4)] The tyramine moiety is also found in most opioids thought to bind specifically with μ-opioid receptor.[3—5)] Morphine-type analgesics are known to acquire narcotic antagonist activity when a group such as an allyl or a cyclopropylmethyl group is introduced onto the nitrogen atom of the tyramine moiety. Kolb[6,7)] considers this to be due to a substitution-induced change in the *N*-lone pair (electron lobe). Thus, the nitrogen atom of the tyramine moiety may be essential for the expression of the agonist and antagonist activities of morphine-type analgesics. Unlike these opioids, however, a group of strong analgesics, 1-substituted 4-[2-(3-hydroxyphenyl)-1-phenylethyl]piperazine derivatives (**1**),[8)] contain two nitrogen atoms in the molecule (Fig. 1). The nitrogen atom at the 4-position (*N*-4) in compound **1** may correspond to the nitrogen atom of the tyramine moiety included in a morphine molecule. However, there is also a possibility that the nitrogen atom of the 1-position (*N*-1) in compound **1** pharmacologically corresponds to the nitrogen atom of the tyramine moiety, since narcotic antagonist activity was induced by introduction of a narcotic antagonist group (*e.g.*, a cyclobutylmethyl group and a 3-methyl-2-butenyl group) onto *N*-1.[9)] Thus, to ascertain roles of these two nitrogen atoms in the expression of opioid agonist and antagonist activities, 4-substituted 1-[2-(3-hydroxyphenyl)-1-phenylethyl]piperidine derivatives (**2**) were synthesized by isosteric substitution[10)] of *N*-1 with a methine (CH) group, and their analgesic action and ability to produce physical dependence as the μ-opioid receptor specific *in vivo* actions,[3)] and narcotic antagonist action in mice were investigated and compared with those of compound **1**.

Chemistry Compounds **2a** and **2b** were synthesized by the routes shown in Chart 1. Racemic **2b** was first prepared as follows. Benzaldehyde and potassium cyanide were reacted with 4-cyclohexylpiperidine (**6**)[11)] according to the method of Goodson and Christopher[12)] to give a phenylacetonitrile derivative (**7**). Then, compound **5b** was prepared by the Grignard reaction from compound **7** and *m*-methoxybenzylmagnesium chloride, and was converted to (±)-**2b** by demethylation with 47% hydrobromic acid. Compounds (−)-**2a**, (+)-**2a**, (−)-**2b**, and (+)-**2b** were prepared as follows. The intermediate, 1,5-dichloro-3-cyclohexylpentane (**4b**), was prepared by catalytic reduction of 1,5-dihydroxy-3-phenylpentane[13)] in the presence of a platinum oxide catalyst followed by chlorination with thionyl chloride. Compounds (−)-**5a** and (−)-**5b** were obtained by reactions of (*R*)-(−)-2-(3-methoxyphenyl)-1-phenylethylamine [(*R*)-(−)-**3**][8)] with 1,5-dihalopentanes (**4a** and **4b**, respectively) in the presence of sodium hydrogen carbonate. Compounds (−)-**5a** and (−)-**5b** were then heated with 47% hydrobromic acid to give (−)-**2a** and (−)-**2b**, respectively. Compounds (−)-**2a** and (−)-**2b** were derived from (*R*)-(−)-**3**, hence they each had the *R*-configuration. Compounds (*S*)-(+)-**2a** and (*S*)-(+)-**2b** were also derived from (*S*)-(+)-**3** by the above two step reactions. The compounds thus synthesized are listed in Tables 1 and 2.

Pharmacological Results and Discussion

Each synthesized compound was subcutaneously injected into mice, and its analgesic activity was examined by the tail flick method.[14)] Narcotic antagonist activity was assessed by the tail flick method using antagonist action to morphine as

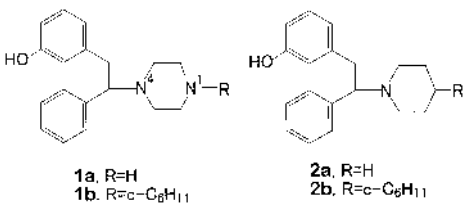

Fig. 1

* To whom correspondence should be addressed.

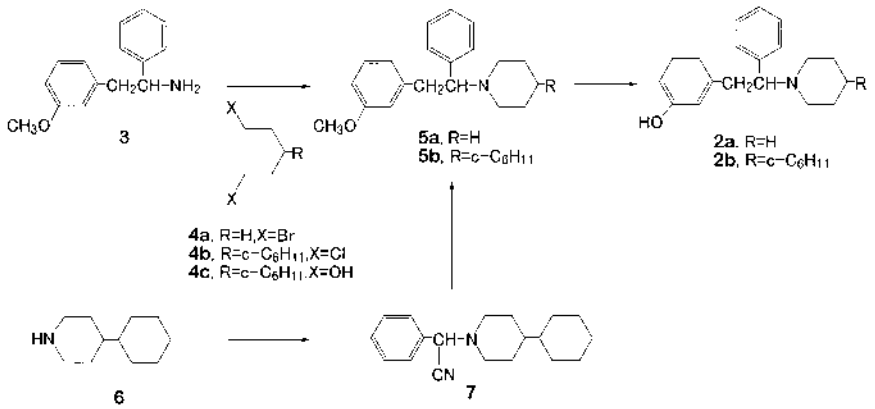

Chart 1

Table 1. 4-Substituted 1-(1,2-Diphenylethyl)piperidine Derivatives

Compd.	X	R	Salt	Procedure[a)]	mp, °C	Recrystn. solvent	Yield %	Formula[b)]
(+)-**2a**	OH	H	HCl	A	220—221	*iso*-PrOH	79	$C_{19}H_{23}NO \cdot HCl$
(−)-**2a**	OH	H	HCl	A	220—221	*iso*-PrOH	76	$C_{19}H_{23}NO \cdot HCl$
(+)-**5a**	OMe	H	HCl	B	253—255	EtOH	52	$C_{20}H_{25}NO \cdot HCl$
(−)-**5a**	OMe	H	HCl	B	254—255	EtOH	54	$C_{20}H_{25}NO \cdot HCl$
(±)-**5b**	OMe	c-C_6H_{11}	HCl	C	217—222	EtOH	60	$C_{26}H_{35}NO \cdot HCl$
(+)-**5b**	OMe	c-C_6H_{11}	Maleate	B	140—142	EtOH	56	$C_{26}H_{35}NO \cdot C_4H_4O_4$
(−)-**5b**	OMe	c-C_6H_{11}	Maleate	B	140—142	EtOH	46	$C_{26}H_{35}NO \cdot C_4H_4O_4$
(±)-**2b**	OH	c-C_6H_{11}	Base	A	160—163	EtOH	86	$C_{25}H_{33}NO$
(+)-**2b**	OH	c-C_6H_{11}	0.5(+)-tartrate	A	196—198	EtOH	83	$C_{25}H_{33}NO \cdot 0.5C_4H_6O_6$
(−)-**2b**	OH	c-C_6H_{11}	0.5(−)-tartrate	A	197—198	MeOH	75	$C_{25}H_{33}NO \cdot 0.5C_4H_6O_6 \cdot 0.5H_2O$

a) Capital letters refer to the procedures in the Experimental Section. *b*) All compounds were analyzed for C, H, N, and, where present, Cl; analytical results were within ± 0.4% of the theoretical values.

an index. The ability to produce physical dependence in mice was assessed by the jumping test[15,16] (Table 3).

As reported previously, among the (*R*)-(−) enantiomers of 1-substituted 4-[2-(3-hydroxyphenyl)-1-phenylethyl]-piperazine derivatives (**1**), some compounds with relatively strong analgesic activity and weak jump-producing activity were antagonistic to opioids. Unlike analgesic activity, antagonist activity toward opioids was observed only for the (*R*)-(−) enantiomers, whereas the (*S*)-(+) enantiomers failed to show such antagonist activity.[8] Analgesic ED_{50}-values of (±)-**1b**, (*S*)-(+)-**1b** and (*R*)-(−)-**1b** have been reported to be 0.126, 0.054 and 4.24 mg/kg, s.c.,[8] and both (+)-**1a** and (−)-**1a** to be more than 80 mg/kg, s.c.[9]

The analgesic potency of (±)-**2b** was 0.39 times that of (±)-**1b** and 10.4 times that of morphine hydrochloride for the molar basis. The analgesic potency of (*S*)-(+)-**2b** was 0.19 times that of (*S*)-(+)-**1b**, whereas (*R*)-(−)-**2b** was 5.34 times more potent than the corresponding (*R*)-(−)-**1b** (Table 3). The potency ratios of the (*S*)-(+) enantiomers to the racemates were 1.94 for (±)-**1b** and 0.95 for (±)-**2b**. Similarly, the ratios of the (*S*)-(+) enantiomers to the (*R*)-(−) enantiomers were 78.6 and 2.81 for 1**b** and **2b**, respectively. Thus, the substitution of *N*-1 with a methine group caused somewhat of a decrease in analgesic activity and an recognizable decrease in enantioselectivity. At a maintenance dose of 1 mg/kg (3.99 mg/kg/2 d), both (±)-**2b** and (*S*)-(+)-**2b** produced jumping in all mice tested (jumping incidence= 100%). Thus, this may show that jumping possibly has parallel analgesic activity, as noted for (±)-**1b** and (*S*)-(+)-**1b** (jump-producing ED50-value: 1.16 and 0.24 mg/kg, s.c., respectively).[8] However, (*R*)-(−)-**2b** showed certain jump-producing activity (Table 3) as did also (*S*)-(+)-**2b**, in contrast to the finding[8] that the maximum jump rate among doses up to 20 mg/kg tested for (*R*)-(−)-**1b** was only 40%. Namely, the jump-producing activity of the racemate and the (*S*)-(+) enantiomer was only slightly affected by the methine-substitution for *N*-1, whereas that of the (*R*)-(−) enantiomer was potentiated by this substitution. Neither (±)-**2b** nor (*S*)-(+)-**2b** showed antagonist action toward opioids, as true in the case of (±)-**1b** and (*S*)-(+)-**1b**. Compound (*R*)-(−)-**1b** was antagonistic to opioids, with maximum reversal rate of 50% at a dose of 2 mg/kg, s.c.,[8] but not (*R*)-(−)-**2b**. Compound (*R*)-(−)-**1a** was clearly antagonistic to opioids, with antagonistic ED_{50}-value of 86.8 mg/kg, s.c.,[9] whereas (*R*)-(−)-**2a** was not (Table 3). Thus, the methine-substitution for *N*-1 abolished the antagonist activity of the (*R*)-(−) enantiomers.

As described above, the isosteric substitution of *N*-1 with a methine group had no significant effect on the analgesic or

Table 2. Data for Optical Rotation

Compd.	Salt	Conf.[a)]	$[\alpha]_D^t$, deg (*c*, t)[b)]
(+)-**2a**	HCl	*S*	+89.1 (1.00, 27)
(−)-**2a**	HCl	*R*	−89.1 (1.00, 27)
(+)-**2b**	0.5(+)-tartrate	*S*	+70.0 (0.50, 26)
(−)-**2b**	0.5(−)-tartrate	*R*	−69.7 (0.50, 26)

a) Absolute configuration. *b*) Solvent: MeOH.

Table 3. Analgesic, Narcotic Antagonist and Jump-Producing Activities of 4-Substituted 1-(1,2-Diphenylethyl)piperidine Derivatives in Mice

Compd.	Salt	ED_{50}, mg/kg, s.c. (95% confidence limits)		
		Analgesic act.	Narcotic antagonist act.	Jump-producing act.
(+)-**2a**	HCl	>80	Inactive	NT
(−)-**2a**	HCl	>80	Inactive	NT
(±)-**2b**	Maleate	0.293 (0.231—0.418)	Inactive	<1 (100%)[a]
(+)-**2b**	0.5(+)-tartrate	0.283 (0.192—1.13)	Inactive	<1 (100%)[a]
(−)-**2b**	0.5(−)-tartrate	0.796 (0.240—1.24)	Inactive	*ca*.1.5
Morphine	HCl	2.39 (1.78—3.20)		5.68 (3.31—9.72)
Pentazocine		>80	3.79 (1.47—9.74)	>80 (17%)[b]

NT: not tested. *a*) Jumping rate (percent of number of mice jumped/tested) at a dosage tested. *b*) Maximum jumping rate (percent of number of mice jumped/tested) among doses tested.

jump-producing activity of the racemate and the (*S*)-(+) enantiomer, whereas it abolished antagonist activity of the (*R*)-(−) enantiomers toward opioids. Thus, the nitrogen atom at the 1-position in the molecule of 1-substituted 4-[2-(3-hydroxyphenyl)-1-phenylethyl]-piperazine derivatives (**1**) possibly contributes to the expression of antagonist action to opioids, and the nitrogen atom at the 4-position may correspond to the nitrogen atom of the tyramine moiety essential for analgesic activity. A relationship between the expression of agonist antagonist activity and receptors for morphine-like analgesics is satisfactorily demonstrated by the above mentioned model of Kolb[6] and the model of Snyder and colleagues.[17] The latter[17] assert that binding subsites may be present on the opiate receptor comprising agonist and antagonist binding sites in addition to an amine binding site. The relation between the two nitrogen atoms in compound **1** appears to support the hypothetical concept of these researchers.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were obtained with a digital polarimeter (Model DIP-4, Japan Spectroscopic Co.,Ltd.). Electron impact mass spectra (EI-MS) were recorded on a Hitachi RMU-6L spectrometer using the direct inlet system at 70-eV ionization potential.

α-(4-Cyclohexylpiperidinyl)phenylacetonitrile (7) This compound was prepared from 4-cyclohexylpiperidine (**6**)[11] in a manner similar to that described in the literature[12]: EI-MS, *m/z* 282 (M^+); Yield 99%.

1,5-Dichloro-3-cyclohexylpentane (4b) This compound was prepared by catalytic hydrogenation (4.0 kg/cm^2) of 1,5-dihydroxy-3-phenylpentane[13] in the presence of a platinum oxide catalyst followed by chlorination with thionyl chloride: bp 125—127 °C (4 mmHg); EI-MS, *m/z* 222 (M^+); Yield 82%.

4-Substituted 1-(1,2-Diphenylethyl)piperidines 2a, 2b, 5a, and 5b (Table 1). Procedure A. (−)-4-Cyclohexyl-1-[2-(3-hydroxyphenyl)-1-phenylethy]piperidine·[(−)-2b] 1/2(−)-Tartrate A mixture of (−)-**5b** (1.5 g, 4.0 mmol), 47% HBr (30 ml), and CH_3COOH (6 ml) was heated at reflux for 1.5 h with stirring. The reaction mixture was made alkaline with 28% ammonium hydroxide and the mixture was extracted with $CHCl_3$. The combined extracts were dried and concentrated to dryness *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$ CH_3OH (30 : 1) to give 1.1 g of (−)-**2b** as an oil. The resulting oily base was converted to its (−)-tartrate with (−)-tartaric acid, and the resulting crystals were recrystallized from C_2H_5OH to give 1.3 g (72%) of (−)-**2b**·1/2(−)-$C_4H_6O_6$·1/2H_2O.

Procedure B. (−)-4-Cyclohexyl-1-[2-(3-methoxyphenyl)-1-phenylethyl]-piperidine[(−)-5b]·Maleate In dimethylformamide (20 ml) were dissolved (*R*)-(−)-2-(3-methoxyphenyl)-1-phenylethylamine [(*R*)-(−)-**3**][8] (2.0 g, 8.8 mmol) and 1,5-dichloro-3-cyclohexylpentane (**4b**) (2.2 g, 9.9 mmol), and $NaHCO_3$ (3.0 g, 36 mmol) was added to the solution. The resulting mixture was heated at reflux for 4 h with stirring and the solvent was removed by distillation *in vacuo*. The reaction products were treated in a manner similar to that described in procedure A. The resulting oily base was converted to its maleate with maleic acid, and the resulting crystals were recrystallized from C_2H_5OH to give 2.0 g (47%) of (−)-**5b**·$C_4H_4O_4$.

Procedure C. (±)-4-Cyclohexyl-1-[2-(3-methoxyphenyl)-1-phenylethyl] piperidine (5b)·Hydrochloride A solution of **7** (4.8 g, 17 mmol) in anhydrous $(C_2H_5)_2O$ (80 ml) was added dropwise with stirring to Grignard reagent which was prepared from magnesium turnings (2.0 g, 0.086 g atoms) and *m*-methoxybenzylchloride (13.4 g, 86 mmol) in anhydrous $(C_2H_5)_2O$ (15 ml), and the mixture was heated at reflux for 3 h. After the reaction mixture was cooled to 0 °C, the remaining Grignard reagent was allowed to decompose by the careful addition of 40 ml of water. The mixture was acidified with concentrated HCl, then cooled and the resulting precipitate was collected by filtration. To the above salt was added 30 ml of 10% NaOH and the mixture was extracted with $CHCl_3$. The combined extracts were washed with water, dried, and concentrated to dryness *in vacuo*. The residue was treated with ethanolic HCl, and the resulting crystals were recrystallized from C_2H_5OH to give 4.2 g (60%) of (±)-**5b**·HCl

Analgesic Assay The compounds listed in Table 3 were tested for analgesic activity by the tail flick method[14] in mice. Three to 4 subcutaneous doses and 5 mice for each dose were used to estimate the analgesic ED_{50} value.

Narcotic-Antagonist Assay[18] The narcotic antagonist ED_{50} value of the compounds was calculated from the number of positive mice showing a response time of less than 10 s in the tail-flick test at 30 or 60 min after a single subcutaneous injection of 5 mg/kg of morphine hydrochloride; this was the injection effective (14—15 s) in prolonging the response time to thermal stimulus in 95% of animals. Each compound was administered subcutaneously just prior to morphine injection. Three to 4 doses and 5 mice for each dose were used to estimate the ED_{50} value.

Physical-Dependence Assay Male mice (19—23 g) of ddN strain were used. Mice were given 7 subcutaneous administrations of each compound during 2 d on an increasing dose schedule until a maintenance dose (after the fifth dose) was reached. Some of the dosage regimens on a regular dose schedule were as follows: 0.08, 0.16, 0.25, 0.5, 1, 1, and 1 mg/kg (total dose=3.99 mg/kg/2 d), and 5, 10, 20, 40, 80, 80, and 80 mg/kg (total dose=315 mg/kg/2 d). Two hours after the last dose, the animals received a single intraperitoneal injection of 50 mg/kg nalorphine hydrochloride, and jumping behavior and other withdrawal signs were observed for 30 min in a separate cylindrical cage (40 cm high and 15 cm in diameter).[15,16] Three to 4 doses and 10 to 20 mice for each dose were used to estimate the ED_{50}, the maintenance dose required to produce 50% jumping of the mice tested.

Analgesic, narcotic antagonist, and jump-producing ED_{50} values and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.[19]

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