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Stereoselective Morphine-Like Discriminative Properties of a New Alkylaminonaphthalenic Derivative

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FRAIOLI, S., L. ANTONILLI AND P. NENCINI. *Stereoselective morphine-like discriminative properties of a new alkylaminonaphthalenic derivative.* PHARMACOL BIOCHEM BEHAV **66**(1) 199–204, 2000.—The morphine-like properties of a series of aminoalkyl- and cycloalkylamino-naphtalenic derivatives of 17-methyl-17-azaequilenine were studied in rats trained to discriminate morphine (5.6 mg/kg IP) from vehicle in a two-lever operant behavioral procedure reinforced by water access. It was found that one of the compounds tested (i.e., A8; 1-ethyl-1-hydroxy-1-[2-(6-hydroxynaphthyl)]-2-methyl-3-dimethylaminopropane) fully generalized for the morphine stimulus. The discriminative effects of A8 were stereospecific, as indicated by the fact that (+)-(1R,2R)-A8 was three times more potent than the racemic compound and that the (−)-(1S,2S) enantiomer was completely inactive. (+)-(1R,2R)-A8 generalization for the morphine cue was inhibited by naloxone. None of the other five derivatives examined generalized for the morphine stimulus. In conclusion, the naphthalenic structure is a source of compounds with stereospecific and naloxone-reversible morphine-like properties. © 2000 Elsevier Science Inc.

Morphine Aminonaphthalenic derivatives Drug discrimination Rats

STRUCTURAL requirements of opioid ligands appear to be quite flexible, as suggested by evidence that compounds with very different geometry possess comparable opioid agonist potency (1,11). Therefore, it is not surprising that even phenolic heterosteroids have been found to cause morphine-like analgesic effects. The most active of these compounds was 17-methyl-17-azaequilenine (10). To test the hypothesis that a less rigid geometry may increment opioid ligand properties of etherosteroids, their aminoalkyl- and cycloalkylamino-naphtalenic derivatives have been developed by Paroli and colleagues (6,7) (Fig. 1). Among them, the alkylaminoalkylnaphthalenic compound 1-ethyl-1-hydroxy-1-[2-(6-hydroxynaphthyl)]-2-methyl-3-dimethylaminopropane (A8) and the cycloaminoalkylnaphthalenic compound 1,2-dimethyl-3-[2-(6-hydroxynaphthyl)]-3-hydroxypyrrolidine (B7) appeared the most potent in terms of analgesic efficacy (3,4). These derivatives did not differ from morphine in their analgesic potency, at least as indicated by the hot-plate test in the mouse. An important aspect of these two molecules is that both of them possess two nonequivalent chiral centers. Therefore, two racemic pairs and four diastereomers are possible for each compound. So far it has been found that (+)-(1R,2R)-A8 is 2.5 times more potent than the racemic compound (1R,2R/1S,2S)-A8 in terms of analgesic activity, whereas the (−)-(1S,2S) enantiomer was completely inactive (3). As far as the B7 compound is concerned, it is the (−)-(2S,3R) enantiomer that shows the highest analgesic activity (4).

In the present study, the morphine-like properties of molecules belonging to both the alkylaminoalkylnaphthalenic and the cycloaminoalkylnaphthalenic series were evaluated by determining their capability to generalize for the morphine stimulus in a drug-discrimination paradigm in the rat. It has been clearly demonstrated that this paradigm is useful for classifying opioid drugs. In particular, rats trained to discriminate morphine from saline generalize for the agonists at mu-opioid receptors, but not for other psychoactive drugs or nonopioid analgesics (2,13). In addition, this generalization is blocked by opioid antagonists and it is stereospecific with only the analgesically active isomer exerting morphine-like effects (2,13).

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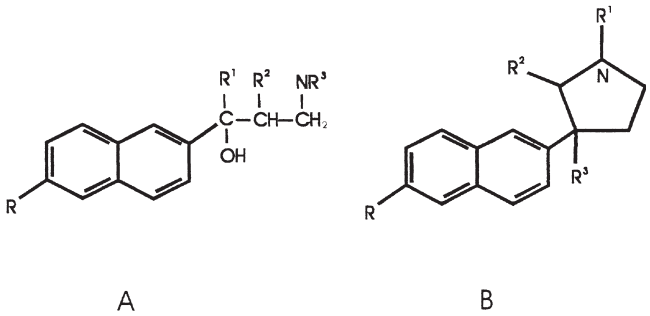

FIG. 1. Molecular structures of aminoalkyl- and cycloalkylaminonaphtalenic compounds (A and B, respectively).

METHOD

Animals

The experimental subjects were 24 male Sprague–Dawley rats, obtained from Morini (San Polo d'Enza, RE, Italy), which weighed 300–380 g at the start of the experiment. Rats were housed individually at constant room temperature (23°C) and humidity, and maintained on a 12 L:12D cycle (0700–1900 h). Experimental procedures were in compliance with the European Communities Council Directive on 24 November 1986 (86/609/EEC).

Drug Discrimination

Morphine vs. vehicle. Twelve rats were trained to discriminate morphine (5.6 mg/kg IP) from vehicle (1 ml/kg IP), as previously described (8). The animals were water restricted and allowed to obtain water by lever-pressing according to a 15-min FR30 schedule of reinforcement in four standard operant cages equipped with two levers. In each chamber one lever was designated the vehicle lever (V) and the other the morphine (M) lever. A training schedule was used in which the vehicle and the drug were administered 10 min before each daily session, according to a semirandom sequence (M, M, V, V, M, V, etc.). Pressing the appropriate lever brought the rat in contact with a dipper containing 0.1 ml of water; pressing the other lever resetted the response requirement for the appropriate lever.

The discrimination criterion required that a rat produced at least 80% of its responses before the first reinforcer on the correct lever for at least seven out of eight consecutive sessions. Test sessions were identical to training sessions except that water was available for responding under an FR30 on either lever and that responding on one lever did not reset the response requirement on the other lever.

Morphine vs. A8 and vehicle. Morphine-like discriminative stimulus properties of (+)-(1R,2R)-A8 were further assessed in an experiment where rats were trained to discriminate A8 from morphine, according to the method described by Overton (9). Twelve rats were initially trained to discriminate vehicle from morphine (5.6 mg/kg IP) as described above. When the FR30 schedule of reinforcement was attained on both levers, rats received periodic injections of (+)-(1R,2R)-A8 (1.7 mg/kg IP) according to the following sequence: S, S, M, M, A, A, M, M, S, S, etc. On days when (+)-(1R,2R)-A8 was administered, only responding on the vehicle lever was reinforced. The training dose of (+)-(1R,2R)-A8 was selected for its morphine-like cueing effects demonstrated in the first experiment.

Data Analysis

For each subject the accuracy of discrimination responses was expressed as the percentage of responses emitted on drug lever. The response rate was calculated as total number of responses/time (responses/s). These data are those shown in the figures. However, to compare discriminative potency of tested compounds, quantal dose–response curves were obtained plotting the number of rats that met the discriminative criterion (80% of responses emitted on the drug-lever during test session) as a function of drug dose (data not shown). The Litchfield–Wilcoxon procedure was then adopted to generate ED_{50} values and 95% confidence intervals (12). Shifts in the dose–effect curves were considered significant if confidence intervals did not overlap.

Drugs

Table 1 shows naphthalenic derivatives designed by E. Paroli and colleagues (6,7), and used in the present study. Racemic A8 ((1R,2R/1S,2S)-1-ethyl-1-hydroxy-1-[2-(6-hydroxynaphthyl)]-2-methyl-3- dimethylaminopropane) and its enantiomers (+)-(1R,2R)-A8 and (−)-(1S,2S)-A8, as well as racemic B7 ((2R,3S/2S,3R)-1,2-dimethyl-3-[2-(6-hydroxynaphthyl)]-3-hydroxypyrrolidine), have been prepared by Ghislandi et al., as previously described (3). The other compounds (i.e., B3, A11, A12, and A13) were prepared by A. Maiorana (Istituto di Chimica Industriale, Università di Milano), as described (6). Morphine hydrochloride was provided by SALARS (Como, Italy), and naloxone hydrochloride by Sigma Chemical Company (St. Louis, MO).

RESULTS

Ten of the 12 rats trained to discriminate morphine (5.6 mg/kg IP) from the vehicle met the discrimination criterion. When generalization tests were performed in these subjects, morphine produced a dose-related increment in drug-appropriate responding, and a progressive decrement in the response rate. Among the naphthalenic derivatives tested, only the A8 compound generalized for morphine stimulus (Figs. 2 and 3). When the dose at which half the subjects generalized for the training drug (ED_{50}) was extrapolated from the quantal dose–response curve, racemic A8 and morphine were demonstrated to be equipotent (Table 2). Figure 3 shows that the other compounds tested did not discriminate for the morphine cue up to the dose of 17.0 mg/kg, but in a different extent they did inhibit response rate.

Morphine-like cueing effects of A8 were stereoselective, with the (+)-(1R,2R) enantiomer being almost three times more potent than the training drug (Fig. 2 and Table 2), whereas the (−)-(1S,2S) enantiomer did not generalize from the morphine stimulus up to the dose of 10 mg/kg. Coadministration of naloxone 0.5 mg/kg shifted to the right the (+)-(1S,2S)-A8 discriminative curve. It is interesting to note that the slope of this curve was abated when 1 mg/kg naloxone was given, suggesting a reduction of A8 efficacy as well as potency (Fig. 2).

The results of the experiment in which the rats were trained to discriminate between A8 and morphine further confirmed the similarities between stimulus properties of (+)-(1S,2S)-A8 and morphine. Figure 4 shows that across 58 sessions the A8 enantiomer generated levels of drug-appropriate responding clearly distinct from that associated with vehicle injection.

TABLE 1

DERIVATIVES OF AMINOALKYL- AND CYCLOALKYLAMINO-NAPHTALENIC STRUCTURES SHOWN IN FIGURE 1

Drug	R	R^1	R^2	R^3
A8	OH	C_2H_5	CH_3	$(CH_3)_2$
A11	OCH_3	$C(CH_3)_3$	H	$(CH_3)_3$
A12	OH	$C(CH_3)_3$	H	$(CH_3)_2$
A13	OH	$C(CH_3)_3$	H	$(CH_3)_3$
B3	OCH_3	CH_3	CH_3	OH
B7	OH	CH_3	CH_3	OH

DISCUSSION

The present study further extends the list of chemical structures with opioid properties, demonstrating that in a series of naphthalenic analogs of 17-methyl-17-azaequilenine, one of the aminoalkyl derivatives possesses morphine-like discriminative stimulus properties. It is thought that drug discrimination represents a reliable model of subjective effects of opioids in humans. In addition, opioid-like discriminative stimulus effects are specific for opioid receptor effects (2,13). First, it is blocked by the administration of opioid receptor antagonists, such as naloxone and naltrexone. Second, when the molecule presents a chiral center, only the stereoisomer with narcotic and analgesic properties generalizes for the opioid stimulus. Both these criteria were met by naphthalenic compound A8, as far as its morphine-like discriminative effects concern. As mentioned above, compound A8 has two nonequivalent chiral centers, and so far, only one pair of stereoisomers, as well as the respective racemate, have been prepared and their analgesic activity investigated. In terms of analgesic effects, the (+)-(1R,2R)-A8 enantiomer was shown to be 2.5 time more potent than the racemate, while analgesic activity was completely absent in the (−)-enantiomer (3). In the present study, we obtained the same potency ratio, although a marginal overlap in the confidence limits of the two

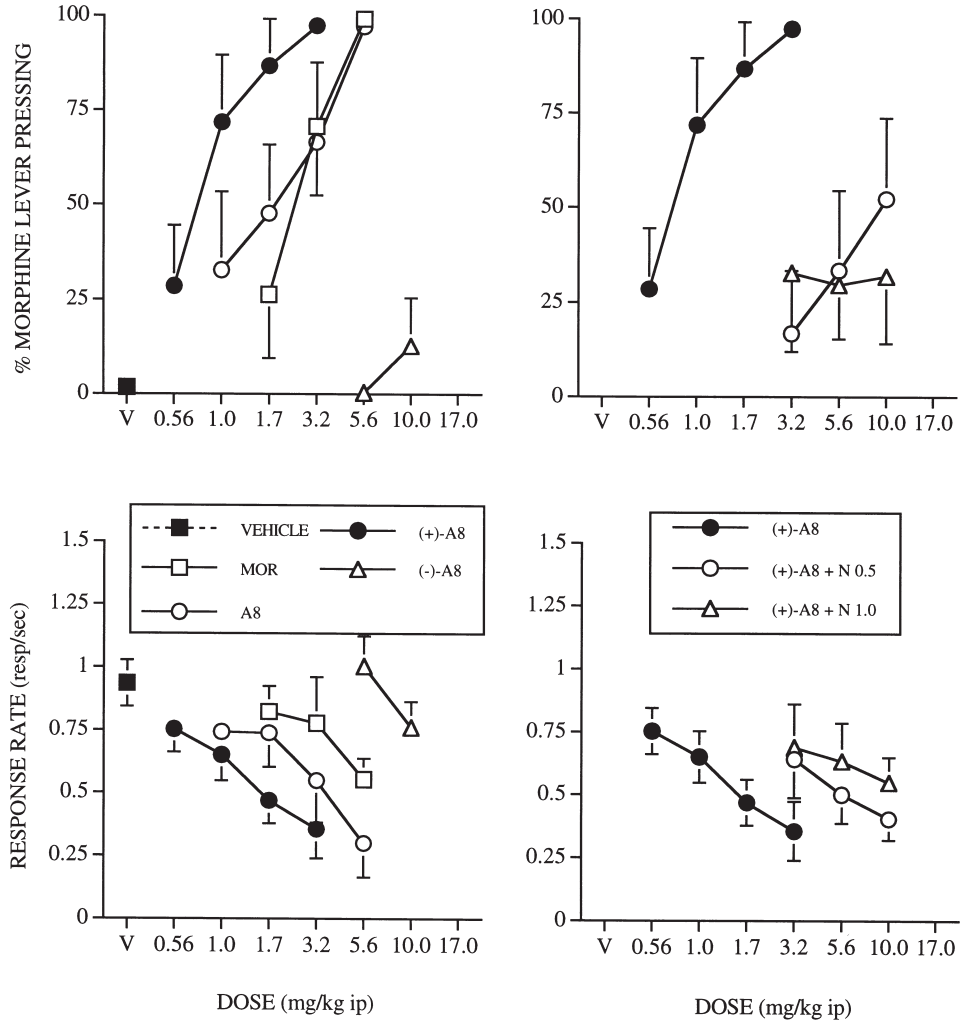

FIG. 2. Effects of morphine, (1R,2R/1S,2S)-A8 (A8), (+)-(1R,2R)-A8 [(+)-A8], and (−)-(1S,2S)-A8 [(−)-A8] on percentage of drug-appropriate responding (left top) and response rate (left bottom) in rats trained to discriminate morphine (MOR, 5.6 mg/kg IP) from vehicle. The right panels show the effects of (+)-(1R,2R)-A8 given alone or in combination with naloxone 0.5 and 1.0 mg/kg IP (N 0.5 and N 1.0) on percentage of drug-appropriate responding (top) and response rate (bottom). The results for testing vehicle are indicated above V. Data are expressed as means + SEM.

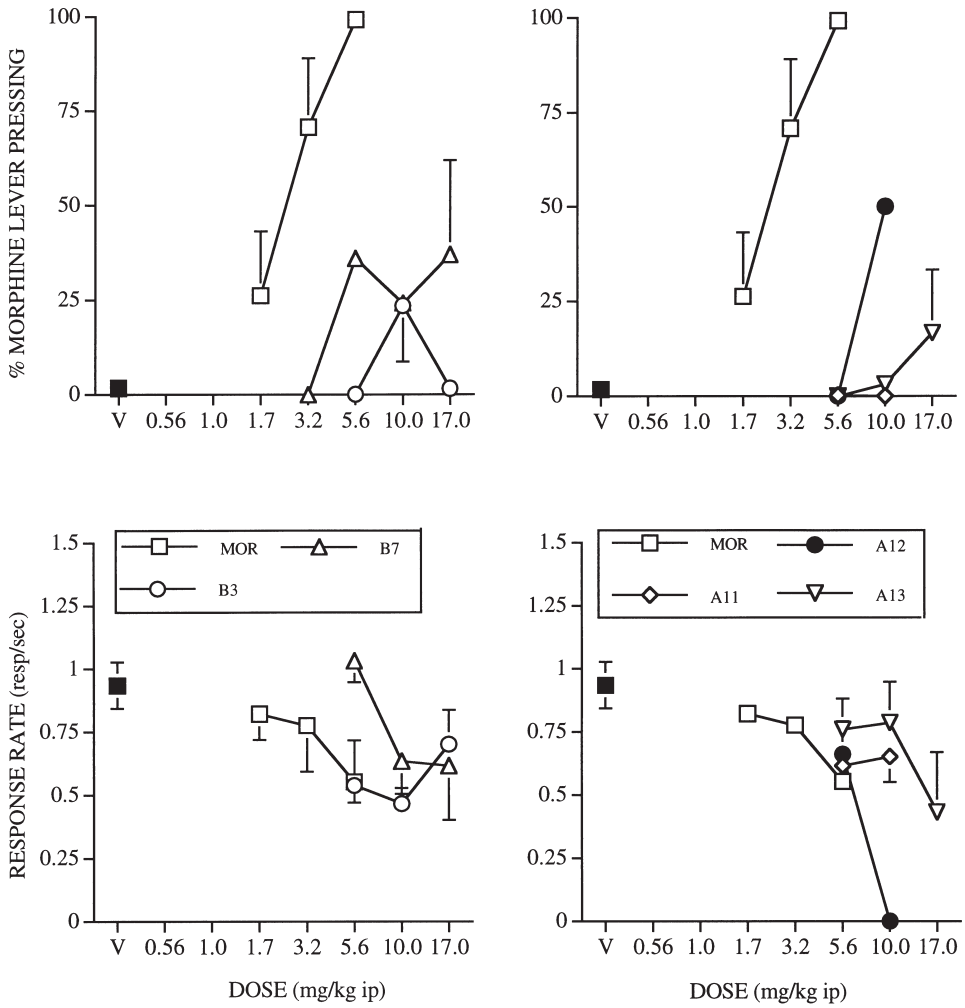

FIG. 3. Effects of morphine, B3 and B7 (left), and A11, A12, and A13 (right) on percentage of drug-appropriate responding (top) and response rate (bottom) in rats trained to discriminate morphine (MOR, 5.6 mg/kg IP) from vehicle.

ED_{50} formally excludes a statistically significant difference between the (+)-(1R,2R)-A8 enantiomer and the racemate. Again, the (−)-(1S,2S) enantiomer was inactive up to a dose 12 times higher than the ED_{50} of (+)-(1R,2R)-A8.

Apparently, the opioid specificity of (+)-(1R,2R)-A8 generalization for the morphine cue was further confirmed by its inhibition by moderate doses of naloxone. However, changes in the slope of the (+)-(1R,2R)-A8 dose–effect curve induced by the higher naloxone dose (1 mg/kg) suggests a noncompetitive antagonism. Thus, (+)-(1R,2R)-A8 generalization for the morphine cue could not be the result of a pure mu-opioid mechanism. The possibility that delta and/or kappa opioid mechanisms contribute to the discriminative cue of the A8 compound deserves further studies. In addition, binding studies, so far lacking, will provide a better profile of this compound, as well as of the other aminonaphthalenic derivatives.

Nevertheless, the experiment in which a different group of animals was trained to discriminate morphine from either vehicle or (+)-(1R,2R)-A8 confirms the similarities of the latter with morphine. By using this procedure, Graham and Balster (5) were able to demonstrate discriminative differences between cocaine and procaine, in the sense that procaine generated responding on the saline lever, whereas in animals trained to discriminate cocaine from saline procaine partially substituted for the drug. On the contrary, in our study rats never learned to discriminate (+)-(1R,2R)-A8 from morphine, confirming the close similarities between the two stimuli.

TABLE 2

QUANTAL ED_{50} VALUES (MG/KG I.P. ± 95% CONFIDENCE INTERVALS) FOR DRUG-APPROPRIATE RESPONDING

	% Morphine Lever Responding	
Drug	ED_{50} (mg/kg)	Confidence intervals
Morphine	2.34	1.73–3.17
(+)-(1R,2R)-A8	0.80	0.55–1.17
(−)-(1S,2S)-A8	>10	–
(1R,2R/1S,2S)-A8	1.99	1.11–3.58

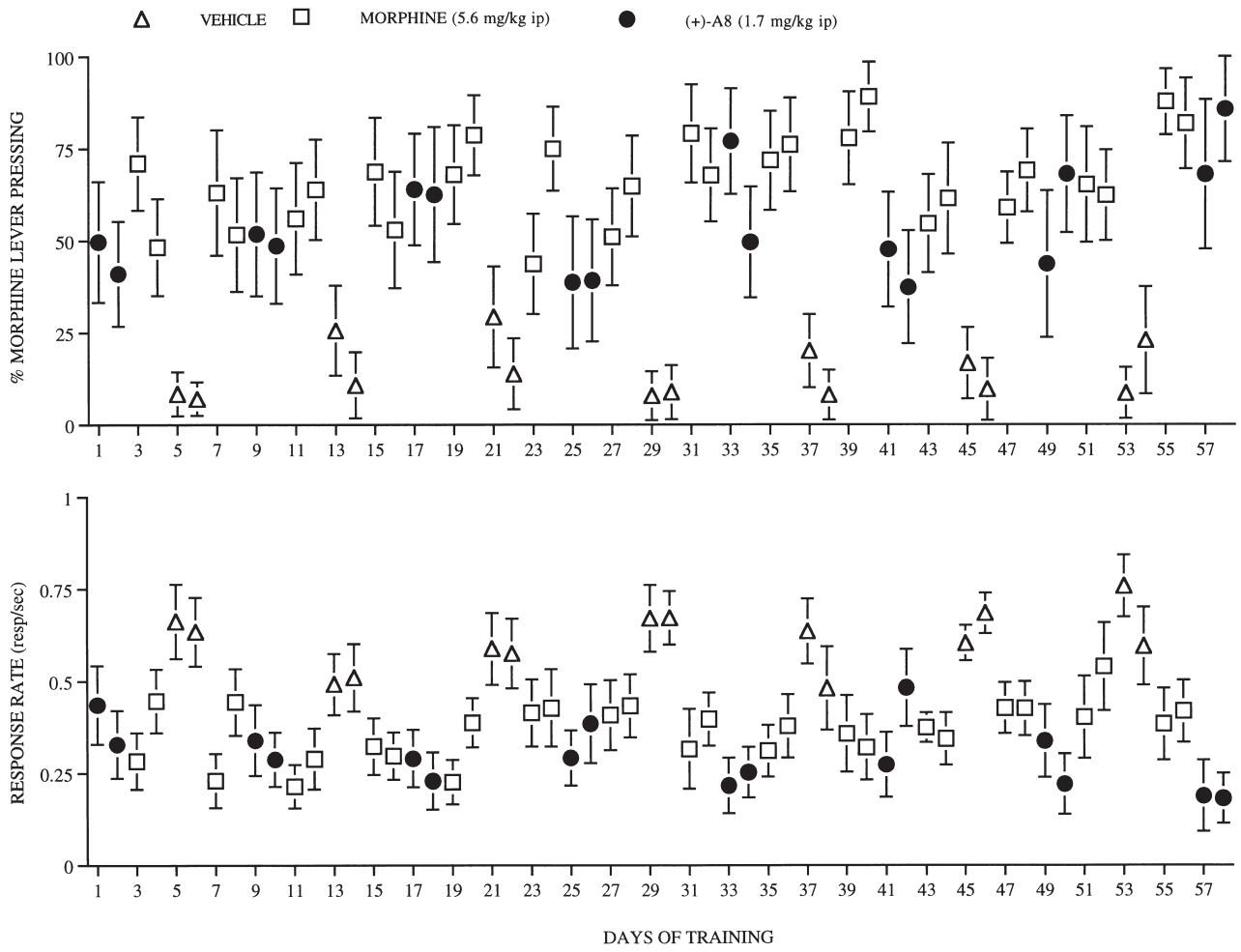

FIG. 4. Percentage of drug-appropriate responding (top) and response rate (bottom) in rats trained to discriminate morphine (all panels) from vehicle or (+)-(1R,2R)-A8 [(+)-A8]. Data are expressed as means + SEM.

Structural requirements to confer morphine-like properties to naphthalenic compounds appeared to be quite strict, as suggested by the loss of activity produced by inserting a bulk substituent at the alpha-carbon atom of the aminoalkyl side chain. Thus, compounds A11, A12, and A13 generated responding on the vehicle lever at doses that substantially reduced response rate. Loss of activity was also detected in compounds where a pyrrolidin moiety was substituted for the aminoalkyl side chain (compounds B3 and B7). In the case of compound B7, our results are apparently at odds with the finding that the compound possesses analgesic properties (4). It is, however, important to observe that these analgesic properties appeared fully expressed when the hot-plate test, but not the writhing test, was used in the mice (4). It is believed that the writhing test, more than the hot plate test, is a model of mu-opioid analgesia (14). Thus, it is possible that cyclization of the aminoalkyl side chain confers specificity for other opioid receptors than mu receptors.

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