cantly more lung tumor nodules than either B16-F1 or B16-F1A9 (Table 1). Also, in spontaneous metastasis assays (*17*) the adapted subline, B16-F1A9, was no more metastatic than the original B16-F1 subline, indicating that mere growth of B16 cells in the lung is insufficient to produce more metastatic cells. Thus, sequential adaptation of tumor cells to an organ environment through a nondestructive method of cell transplantation yields results similar to those of other procedures. These data do not support an adaptation theory for successful metastatic colonization.

Raz *et al.* (*18*), who utilized an ultraviolet radiation–induced fibrosarcoma, came to the same conclusion. By intravenously injecting fibrosarcoma cell clones with low lung colonization potential, harvesting the few lung tumor nodules that formed, culturing these in vitro, and retesting their lung colonization potentials, Raz *et al.* found that one cycle of tumor cell growth in the lung is not sufficient to increase the potential of a given cell line to form pulmonary metastases. In addition, Raz *et al.* found that the potential of parental fibrosarcoma cells to spread to the lung is enhanced when they are injected intraperitoneally, harvested, and grown in vitro.

Thus, it appears that tumor cells adapted to the lung do not have an increased ability to spread to that site. Metastasis is not an adaptive process; instead, it appears to involve selection of preexistent tumor cells with higher metastatic potential (*1, 6, 7, 14, 18*).

GARTH L. NICOLSON
SUSAN E. CUSTEAD
Department of Tumor Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston 77030

References and Notes

1. I. J. Fidler, D. M. Gersten, I. R. Hart, *Adv. Cancer Res.* **28**, 149 (1978); E. V. Sugarbaker, *Curr. Probl. Cancer* **3**, 3 (1979); G. L. Nicolson, *BioScience* **28**, 441 (1978); *Sci. Am.* **240**, 66 (March 1979); G. Poste and I. J. Fidler, *Nature (London)* **283**, 139 (1980); I. J. Fidler and G. L. Nicolson, *Cancer Biol. Rev.* **2**, 174 (1981).
2. A. M. Cloudman, *Cancer Res.* **7**, 585 (1947); E. V. Sugarbaker, *Cancer (Philadelphia)* **5**, 606 (1952); B. Lucke, C. Breedis, Z. P. Woo, L. Berwick, P. Nowell, *Cancer Res.* **12**, 734 (1952); T. B. Dunn, *J. Natl. Cancer Inst.* **14**, 1281 (1954); D. L. Kinsey and R. R. Smith, *Surg. Gynecol. Obstet.* **109**, 539 (1959); H. S. N. Green and E. K. Harvey, *Cancer Res.* **24**, 799 (1964); I. J. Fidler and G. L. Nicolson, *J. Natl. Cancer Inst.* **57**, 1199 (1976); *ibid.* **58**, 1867 (1977); V. Schirrmacher, G. Shantz, K. Claver, D. Komitowski, H.-P. Zimmermann, M.-L. Lohmann-Matthes, *Int. J. Cancer* **23**, 233 (1979); A. Neri, D. Welch, T. Kawaguchi, G. L. Nicolson, *J. Natl. Cancer Inst.*, in press.
3. S. Paget, *Lancet* **1889-I**, 571 (1889).
4. E. Klein, *Exp. Cell Res.* **8**, 88 (1955)
5. I. J. Fidler, *Nature (London)* **242**, 148 (1973); K. W. Brunson, G. Beattie, G. L. Nicolson, *ibid.* **272**, 543 (1978); K. W. Brunson and G. L. Nicolson, *J. Natl. Cancer Inst.* **61**, 1499 (1978); *J. Supramol. Struct.* **11**, 517 (1979); T.-W. Tao, A. Matter, K. Vogel, M. M. Burger, *Int. J. Cancer* **23**, 854 (1979), R. W. Kerbel, R. R. Twiddy, D. M. Robertson, *ibid.* **22**, 583 (1978); P. J. Shearman and B. M. Longenecker, *ibid.* **24**, 363 (1980); I. R. Hart, J. E. Talmadge, I. J. Fidler, *Cancer Res.* **41**, 1281 (1981).
6. I. J. Fidler and M. L. Kripke, *Science* **197**, 893 (1977).
7. G. L. Nicolson, K. W. Brunson, I. J. Fidler, *Cancer Res.* **38**, 4105 (1978); N. Suzuki, H. R. Withers, M. W. Koehler, *ibid.*, p. 3349; M. L. Kripke, E. Gruys, I. J. Fidler, *ibid.*, p. 2962; J. E. Talmadge, J. R. Starkey, W. C. Davis, A. L. Cohen, *J. Supramol. Struct.* **12**, 277 (1979); A. F. Chambers, R. P. Hill, V. Ling, *Cancer Res.* **41**, 1368 (1981); D. A. Chow and A. M. Greenberg, *Int. J. Cancer* **25**, 261 (1980); I. J. Fidler, E. Gruys, M. A. Cifone, Z. Barnes, C. Bucana, *J. Natl. Cancer Inst.* **67**, 947 (1981).
8. G. H. Heppner, D. L. Dexter, T. DeNucci, F. R. Miller, P. Calabresi, *Cancer Res.* **38**, 3758 (1978); R. Lotan and G. L. Nicolson, *ibid.* **39**, 4767 (1979); A. Biorklung, L. Hakansson, B. Stenstam, C. Tropes, M. Akerman, *Br. J. Cancer* **16**, 647 (1980); G. L. Nicolson, R. Lotan, A. Rios, *Cancer Treat. Rep.*, in press; T. Tsuruo and I. J. Fidler, *Cancer Res.* **41**, 3058 (1981).
9. L. Revesz and L. Norman, *J. Natl. Cancer Inst.* **25**, 1041 (1963); H. Z. Hill, G. J. Hill, C. F. Miller, F. Kwong, J. Purdy, *Radiat. Res.* **80**, 259 (1979).
10. S. P. Tomasovic, H. D. Thames, G. L. Nicolson, *Radiat. Res.*, in press.
11. T.-W. Tao and M. M. Burger, *Nature (London)* **270**, 437 (1977); C. L. Reading, P. N. Belloni, G. L. Nicolson, *J. Natl. Cancer Inst.* **64**, 1241 (1980); J. W. Dennis, T. B. Donaghue, R. S. Kerbel, *ibid.* **66**, 129 (1981).
12. M. V. Pimm and R. W. Baldwin, *Int. J. Cancer* **20**, 37 (1977); R. S. Kerbel, *Am. J. Pathol.* **97**, 609 (1979); L. Olsson and P. Ebbesen, *J. Natl. Cancer Inst.* **62**, 623 (1979).
13. V. S. Byers and J. O. Johnson, *Cancer Res.* **37**, 3173 (1977); G. Goldin and R. K. Johnson, in *Recent Advances in Cancer Treatment*, H. J. Tagnon and M. J. Staquet, Eds. (Raven, New York, 1977), p. 155; C. L. Reading, K. W. Brunson, M. Torriani, G. L. Nicolson, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5943 (1980).
14. K. W. Brunson and G. L. Nicolson, in *Brain Metastasis*, L. Weiss, H. Gilbert, J. B. Posner, Eds. (Hall, Boston, 1980), p. 50.
15. The Bio-Carrier beads (Bio-Rad Laboratories) were added to a solution containing 0.1*M* NaCl and 50 m*M* Hepes buffer (20 g of beads per liter) and the *p*H was adjusted to 6.1. After stirring, the *p*H was adjusted to 6.4 and the suspension was poured into small tubes and autoclaved at 121°C and 15 pounds per square inch for 20 minutes with low exhaust. Sterile beads were stored at 4°C until use. B16 melanoma sublines were grown to confluence in 100-mm-diameter tissue culture plates in a 1:1 mixture of Eagle's minimum essential medium (Dulbecco's modification) and F12 medium supplemented with 10 percent fetal bovine serum (Flow Laboratories). Cell cultures were rinsed twice in medium and 1 ml of the bead-containing suspension was added with fresh medium and 10 percent serum. After 2 days the beads were removed by gently washing the cell monolayers with phosphate-buffered saline free of calcium and magnesium ions. The beads were allowed to settle and were resuspended in fresh medium without serum at a concentration of approximately 150 beads per milliliter. This suspension was quickly injected intravenously into 8-week-old female C57BL/6 mice (0.2 ml per animal).
16. I. Zeidman and J. M. Buss, *Cancer Res.* **12**, 731 (1952).
17. I. J. Fidler, *ibid.* **35**, 218 (1975). Mice were injected subcutaneously with 5×10^5 viable tumor cells. After 2 weeks the resultant subcutaneous tumors were excised, and 8 weeks later the animals were killed and examined. Some of the ten mice injected with subline B16-F1 developed spontaneous metastases (two mice had lung tumors, three had lymph node tumors, and one had abdominal tumors). Similarly, of ten mice injected with subline B16-F1A9, one had lung tumors and two had lymph node tumors.
18. A. Raz, N. Hanna, I. J. Fidler, *J. Natl. Cancer Inst.* **66**, 183 (1981).
19. We thank R. Davis, K. Dulski, J. Smith, and M. Torrianni for their assistance. This work was supported by National Cancer Institute grant RO1-CA28867.

12 August 1981; revised 28 September 1981

Stereoisomers of *N*-Allylnormetazocine: Phencyclidine-Like Behavioral Effects in Squirrel Monkeys and Rats

Abstract. *(±)-N-Allylnormetazocine is a benzomorphan opioid with psychotomimetic effects. The pure stereoisomers of this compound, as well as the racemic mixture, were compared to phencyclidine for their behavioral effects on squirrel monkeys and rats trained to discriminate phencyclidine from saline. Dose-response determinations were made for responses to phencyclidine, to a racemic mixture of N-allylnormetazocine, and to the pure levo and dextro isomers of N-allylnormetazocine. In both rats and monkeys, the dextro isomer and the racemic mixture produced dose-dependent responses appropriate for phencyclidine; the levo isomer did not produce the responses appropriate for phencyclidine at any of the doses tested. In both species, the levo isomer was more potent than the dextro isomer in decreasing the rate of responding. Thus racemic N-allylnormetazocine is a mixture of compounds that produce different behavioral effects.*

The pharmacological properties of the dissociative anesthetics, phencyclidine (PCP) and ketamine, appear to overlap those of the psychotomimetic opioids. The effects of PCP on the dog with transected spinal cord (*1*) are similar to the effects of (±)-*N*-allylnormetazocine (SKF 10,047), a benzomorphan opioid considered to be the prototypical agonist of the putative σ opiate receptor (*2*). Rats trained to discriminate PCP from saline generalize the PCP response to a series of structural analogs of PCP and to (±)-*N*-allylnormetazocine but not to some other psychoactive substances (*3*). Rats and pigeons trained to discriminate cyclazocine, another psychotomimetic benzomorphan, from saline generalize the cyclazocine response to PCP, ketamine, and dextrorphan (*4*). Rhesus monkeys generalize their responses to ketamine to (±)-*N*-allylnormetazocine and dextrorphan, but not to cyclazocine nor to the levo (−) isomer of dextrorphan, levorphanol (*5*). Because the dissociative anesthetic–like effects of some opioids

0036-8075/82/0108-0178$01.00/0

may be stereospecific, we have examined the responses to *N*-allylnormetazocine, both as the racemic mixture and as the pure stereoisomers, in rats and squirrel monkeys trained to discriminate between PCP and saline. Since cyclazocine appears to produce effects similar to those of dissociative anesthetics in the rat but not in the rhesus monkey, we studied a rodent and a primate species. We now report that the PCP-like properties of racemic *N*-allylnormetazocine in both species are more specific to the pharmacological activity of the dextro (+) isomer.

Adult male rats (Sprague-Dawley) and squirrel monkeys were deprived of food until they attained 80 percent of their weights when given free access to food. They were trained in operant chambers to press one of two response levers (*6*) on a fixed ratio 32 (FR32) schedule of food presentation (*7*) during daily 30-minute sessions. During the training period, the animals were given an injection of physiological saline or PCP; for rats, the dose was 3.0 mg/kg intraperitoneally 10 minutes before each session, and for monkeys, the dose was 0.16 mg/kg intramuscularly 5 minutes before each session. Two days of PCP injection were alternated with 2 days of saline injection. For each animal, responding on one of the levers was reinforced on days when drug was given and responding on the other lever was reinforced on days when saline was given. Responses on the incorrect lever reset the contingency for reinforced responding on the correct lever. Every third session began with a 2-minute period during which responding on either lever was reinforced (rats) or responding on neither lever was reinforced (monkeys); the session then continued as usual on the FR32 schedule. Discrimination training was continued until a subject had ten consecutive test periods with at least 80 percent of the responses on the appropriate lever.

After reliable discrimination was established, test sessions with treatments other than those of the training conditions were begun. Test sessions consisted of a 2-minute period during which a response on either lever was reinforced under the FR32 schedule (rats) or the response was recorded but not reinforced (monkeys). The sessions were then ended, and the animals were returned to their cages. A dose-response determination for responses to PCP was completed first in each animal. Then testing was conducted with doses of (±)-*N*-allylnormetazocine, (+)-*N*-allylnormetazocine, and (−)-*N*-allylnormetazocine chosen to range from having no effect to producing nearly complete suppression of responses (*8*). The stereoisomers of *N*-allylnormetazocine were prepared by stereospecific synthesis (*9*). After dose-response determinations were made for all four compounds, the interaction of naloxone with a selected dose of each isomer of *N*-allylnormetazocine was assessed in each animal (*10*).

Overall response rate on both levers and the proportion of responses on the lever appropriate for PCP were analyzed for the test days (*11*). The median effective dose (ED_{50}) for each drug in each species was determined by least-squares analysis of the dose-response data for the linear portions of the dose-response curves (*12*).

In both species, 90 to 95 percent of the responses to the training dose of PCP were made on the lever appropriate for drug (Fig. 1). Higher doses of PCP produced a higher percentage of responses on the lever appropriate for drug and lower doses of PCP produced fewer responses on the lever appropriate for drug in a dose-dependent manner. Both (±)-*N*-allylnormetazocine and (+)-*N*-allylnormetazocine produced a dose-related increase in the percentage of responses made on the lever appropriate for PCP. At some doses, both compounds produced stimulus control of responding that was comparable to or greater than that obtained with the training dose of PCP. With the (−)-isomer, no more than 21 percent of the responses in either species were on the lever appropriate for PCP.

All four compounds produced a dose-dependent decrease in the rate of re-

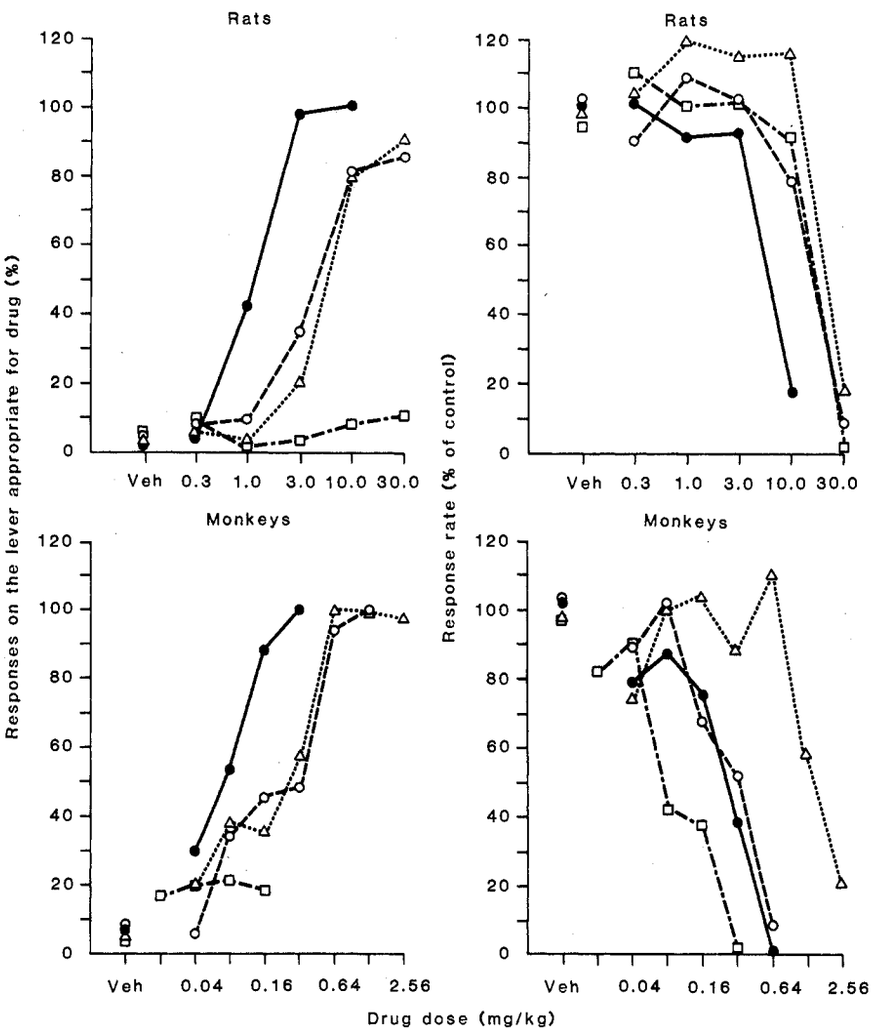

Fig. 1. Dose-response curves for generalization to the PCP cue (left panels) and for effects on overall response rates (right panels) for (●) PCP, (○) (±)-*N*-allylnormetazocine, (△) (+)-*N*-allylnormetazocine, and (□) (−)-*N*-allylnormetazocine for rats (upper panels) and squirrel monkeys (lower panels). Data are averages for all of the tests in all of the animals (when the overall response rate was below 0.5 response per second, the data for that subject was not included in the calculation of percentage of responses appropriate for drug). Each point represents one or two determinations (*11*) in each of 12 rats or 6 monkeys. *Veh*, vehicle.

sponding. Doses of PCP and (+)-*N*-allylnormetazocine could be found for which more than 80 percent of the responses were on the lever appropriate for drug, without marked reduction of response rates. Doses of the racemic mixture that produced the same effects as PCP caused the overall response rate to decrease, particularly in monkeys.

Potency comparisons were made for each drug with doses estimated to produce responses appropriate to the PCP lever 50 percent of the time and with doses necessary to decrease response rates to 50 percent of control values, as determined by the regression analyses. The (+)-isomer and the racemic mixture were about equally potent in producing responses appropriate for PCP (about half as potent as PCP in the squirrel monkey and one-fourth as potent as PCP in the rat). Since the (−)-isomer did not produce the response appropriate for PCP in either species, the racemic mixture might have been expected to be half as potent as the (+)-isomer, since the racemic compound is a mixture of compounds that are active and inactive in producing the PCP cue. Why such an additive effect is absent is not clear.

For decreasing the rate of responding, (±)-*N*-allylnormetazocine has a potency intermediate between those of the (+)- and (−)-isomers, in both rats and squirrel monkeys. The difference in potency of the isomers was particularly striking in the monkeys, where the (−)-isomer was 18 times more potent than the (+)-isomer and was three times more potent than PCP. All of the drugs that produced PCP-appropriate responses were two to five times more potent in the monkey in producing the PCP-like effects than they were in decreasing the overall response rate. The (+)-isomer showed a greater separation of these effects than the racemic mixture did, probably because of the potent response disruption properties of the (−)-isomer in the mixture. In the rat, PCP, the racemic mixture, and the (+)-isomer of *N*-allylnormetazocine were all about three times more potent in producing lever responses appropriate for drug than they were in reducing the response rates.

High doses of naloxone tested in both species did not antagonize the PCP-appropriate responding produced by (+)-*N*-allylnormetazocine or the reduction of the response rate produced by (−)-*N*-allylnormetazocine. These doses of naloxone when given alone did not produce responses appropriate for PCP or decrease the response rates.

The results in both squirrel monkeys and rats are consistent in showing that the PCP-like stimulus properties of (±)-*N*-allylnormetazocine in rats (*3*) is more specific to the (+)-isomer. Although the (−)-isomer produces behavioral activity, this activity is not similar to that produced by PCP. Like other racemic benzomorphans (*13*), (±)-*N*-allylnormetazocine is a mixture of drugs with differing activity and thus it may interact with more than one receptor. The separation of activity was most striking in the squirrel monkey in that the (−)-isomer did not produce PCP-like effects, but was nearly 20 times more potent than the (+)-isomer in decreasing the overall response rate. The (−)-isomer is a nalorphine-like antagonist that will precipitate withdrawal in the morphine-dependent rhesus monkey, whereas the (+)-isomer neither precipitates withdrawal nor supports morphine dependence (*14*). Naloxone did not antagonize the effects of either isomer. Neither naloxone nor naltrexone block the effects of the racemic mixture on schedule-controlled behavior in rats (*15*). Thus, although the (−)-isomer clearly has narcotic antagonist properties, other effects of the isomers of *N*-allylnormetazocine are probably not opioid.

These findings have a bearing on reports (*16, 17*) of a specific binding site for PCP in the rodent nervous system. (±)-*N*-Allylnormetazocine and structural analogs of PCP bind to this receptor, and their relative affinities for binding correlate well with their relative potencies in cross generalization to PCP in the drug discrimination model (*16*). Behavioral and pharmacological work with the pure optical isomers of *N*-allylnormetazocine can help to characterize the σ opiate receptor and the putative PCP receptor and to explore further the similarities between the psychotomimetic opiates and the dissociative anesthetics.

KATHLEEN T. BRADY
ROBERT L. BALSTER
EVERETTE L. MAY

Pharmacology Department,
Medical College of Virginia,
Virginia Commonwealth University,
Richmond 23298

References and Notes

1. D. B. Vaupel and D. R. Jasinski, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 1094 (1979).
2. W. R. Martin, C. G. Eades, J. A. Thompson, R. E. Huppler, P. E. Gilbert, *J. Pharmacol. Exp. Ther.* **197**, 517 (1976).
3. S. G. Holtzman, *ibid.* **214**, 614 (1980); H. E. Shannon, *ibid.* **216**, 543 (1981).
4. J. J. Teal and S. G. Holtzman, *ibid.* **212**, 368 (1980); S. Herling, E. H. Coale, Jr., D. W. Hein, G. Winger, J. H. Woods, *Psychopharmacology* **73**, 286 (1981).
5. A. M. Young, S. Herling, G. D. Winger, J. H. Woods, *NIDA Res. Monogr. Ser.* **34**, 173 (1981).
6. For a detailed description of the squirrel monkey apparatus, see K. T. Brady and R. L. Balster [*Pharmacol. Biochem. Behav.* **14**, 213 (1981)]. The monkeys were seated in a plastic chair facing a wall that had two response levers at equal distances to the right and left above a food trough. For the rat study, a standard two-lever operant chamber was housed inside a light- and sound-attenuating cubicle. The levers were 10 cm from the floor of the chamber at equal distances to the right and left above a food cup. A house light was located 20 cm above the food cup.
7. Thirty-two responses were required for each food presentation.
8. The order in which the drugs were tested in each subject was randomized. All doses of one drug were administered to a given subject before the next drug was tested. For the rats, all doses were tested twice in each subject. For the monkeys, the three middle doses of each drug were tested twice. When doses were tested twice, one test followed a PCP training day and one test followed a vehicle training day. Test sessions were conducted only if the subject completed the first fixed ratio on the appropriate lever on the training day preceding the test day. At least two training sessions occurred between each test session.
9. (±)-2-Allyl-5,9α-dimethyl-2′-hydroxy-6,7-benzomorphan or α-(±)-*N*-allylnormetazocine [M. Gordon, J. J. Lafferty, D. H. Tedischi, N. B. Eddy, E. L. May, *Nature (London)* **192**, 1099 (1961)] was prepared by reacting (±)-normetazocine with one equivalent of allyl bromide in a refluxing mixture (8 ml/g) of dimethylformamide and tetrahydrofuran (2:1) in the presence of excess $NaHCO_3$ for 3 to 5 hours. The α-(±)-*N*-allylnormetazocine was isolated as the hydrochloride ($C_{17}H_{23}NO \cdot HCl$, melting point 219° to 220°C) after recrystallization from a mixture of methanol and acetone. Similarly the enantiomers α-(−)- and α-(+)-*N*-allylnormetazocine were obtained from (−)- and (+)-normetazocine and isolated as the hydrobromide salts, which were crystallized from acetone as the hemihydrates ($C_{17}H_{23}NO \cdot HBr \cdot \frac{1}{2}H_2O$). The (−)-hydrobromide melted at 183° to 184°C; $[\alpha]_D^{23}$, −76.9 degrees (c 1.012, water). The (+)-hydrobromide melted at 182° to 184°C; $[\alpha]_D^{25}$, +76.5 degrees (c 0.536, water). The free bases melted at 138° to 139°C and had $[\alpha]_D^{25}$, −121.6 and +121.3 degrees (c 0.920, 1.022, respectively, methanol). Composition of all three salts was confirmed by C, H, N analysis and high-resolution mass spectrometry. Purity was assured by thin-layer chromatography on silica gel.
10. Two-minute test sessions were conducted as described. Rats were tested with naloxone hydrochloride, 30 mg/kg subcutaneously 20 minutes before the session, in combination with (+)- and (−)-*N*-allylnormetazocine, 10 mg/kg intraperitoneally 10 minutes before the session. Monkeys were tested with naloxone hydrochloride, 1.0 and 3.0 mg/kg, in combination with (+)-*N*-allylnormetazocine, 0.64 mg/kg, and (−)-*N*-allylnormetazocine, 0.32 mg/kg; all injections were given intramuscularly 5 minutes before the session.
11. A saline test session conducted at the end of each individual drug dose-response determination was used to calculate vehicle response rates and the percentage of responses appropriate to the drug lever after vehicle administration. Drug lever responding was calculated by dividing the number of responses on the drug lever by the total number of responses on both levers during the test period. Effects of treatments on overall response rates were calculated as the percent of the response rate on the vehicle test days conducted for each drug.
12. Regression analyses were carried out following $\log_{10}$ transformation of the dose expressed as amount of free base.
13. J. E. Villarreal, in *Drug Dependence*, R. T. Harris *et al.*, Eds. (Univ. of Texas Press, Austin, 1970), pp. 83–116.
14. H. H. Swain, J. E. Villarreal, M. H. Seevers, *Proc. Comm. Probl. Drug Dependence* **3**, 698 (1973).
15. R. A. Harris, *J. Pharmacol. Exp. Ther.* **213**, 497 (1980).
16. S. R. Zukin and R. S. Zukin, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5372 (1979).
17. J. P. Vincent, B. Kartalovski, P. Geneste, J. M. Kamenka, M. Lazdunski, *ibid.*, p. 4678; R. Y. Hampton, F. Medzihradsky, J. H. Woods, *Pharmacologist* **22**, 285 (1980).
18. We are grateful to H. Merz, C. W. Boehringer Sohn, Ingelheim Am Rheim, West Germany, for supplies of the normetazocines and to A. E. Jacobson, National Institutes of Health, for analytical and mass spectral data. Supported by National Institute of Drug Abuse (NIDA) grants DA-01442 and DA-00490; K.T.B. was an NIDA predoctoral trainee.

23 March 1981; revised 14 July 1981