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The Effect of Amfonelic Acid or Nisoxetine in Combination With Morphine on Brain-Stimulation Reward

SARI IZENWASSER[1] AND CONAN KORNETSKY[2]

Laboratory of Behavioral Pharmacology, Boston University School of Medicine
80 East Concord Street, Boston, MA 02118

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IZENWASSER, S. AND C. KORNETSKY. *The effect of amfonelic acid or nisoxetine in combination with morphine on brain-stimulation reward.* PHARMACOL BIOCHEM BEHAV **32**(4) 983–986, 1989.—Many drugs of abuse, including stimulants such as cocaine and amphetamine, and opioids like morphine and heroin, will lower the threshold at which rats will work to receive electrical stimulation to the medial forebrain bundle-lateral hypothalamic region (MFB-LH). This effect is even greater when the two classes of drugs are coadministered. The underlying mechanisms by which this occurs are not completely understood, however there is considerable evidence suggesting that the catecholamines play a major role in mediating the reinforcing effects of these drugs. The present study was conducted to investigate the effects of amfonelic acid, an indirect dopamine agonist, and nisoxetine, a highly selective norepinephrine uptake blocker, alone and in combination with morphine, on the reward threshold for rewarding electrical intracranial stimulation. As in previous studies, morphine, as well as amfonelic acid, lowered the reward threshold with the amfonelic acid causing greater threshold lowerings than that of morphine. When a low (ineffective) dose of amfonelic acid was administered concomitantly with morphine, the threshold lowerings observed were larger than those seen with either drug alone and were often more than additive. Nisoxetine alone had no effect on the reward threshold and produced inconsistent results when combined with morphine. These findings support the thesis that amfonelic acid has abuse potential, and that its reinforcing effects may, in fact, be even greater than that of the opioids. Further, these results support the hypothesis that dopamine plays a more critical role in mediating brain-stimulation reward than dose norepinephrine.

Brain-stimulation reward Amfonelic acid Nisoxetine Morphine

IT has been suggested by a number of investigators that the reinforcing effects of a variety of abuse substances, specifically the psychomotor stimulants and the opioids, are a direct result of the activation of those brain areas that subserve intracranial self-stimulation (ICS) (13). Psychomotor stimulants, such as cocaine (10) and d-amphetamine (11), which increase activity of catecholamines at postsynaptic receptors, lower the threshold and increase response rate for ICS (35). Conversely, drugs which antagonize catecholamine activity attenuate ICS behavior. For example, haloperidol (9), chlorpromazine (12) and pimozide (5) raise the threshold (5,9) or decrease response rate for ICS (38,40). Similarly, synthesis inhibition by alpha-methyl-p-tyrosine (37) and lesions of terminal areas by 6-OHDA (6) attenuate ICS behavior.

Like the stimulants, opiates such as morphine (24) and heroin (16) lower the reward threshold. Whether there is a common pathway by which these effects are produced, or whether the two classes of drugs act via different mechanisms to produce reinforcement, is unknown. Although the opiate antagonist naloxone blocks the threshold lowering effects of cocaine (3) and d-amphetamine (11), and the combined administration of d-amphetamine and morphine results in a greater threshold lowering than seen with either drug alone (17), evidence suggesting a different mechanism is given by the finding that 6-OHDA lesions of the nucleus accumbens will block the self administration of cocaine, but not heroin (28). However, clinical subjects to whom combinations of d-amphetamine and morphine were administered report a greater degree of euphoria than with either drug alone (18). Although these findings suggest an interaction between opioid and catecholaminergic systems in mediating central reward, it is difficult to draw conclusions about the mechanism by which this potentiation occurs because d-amphetamine does not act selectively on a single neurotransmitter system.

The present study was conducted to investigate the roles that

[1]Present address: Sari Izenwasser, Ph.D., Department of Pharmacology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

[2]Requests for reprints should be addressed to Conan Kornetsky, Ph.D., Boston University School of Medicine, 80 East Concord Street, L-602, Boston, MA 02118.

norepinephrine and dopamine play in mediating the d-amphetamine-morphine interaction. The effects of amfonelic acid and nisoxetine alone and in combination with morphine on brain-stimulation reward were studied. Amfonelic acid is an indirect dopamine agonist which facilitates the release of dopamine from the presynaptic neuron (32). It will also by itself lower the threshold for rewarding brain stimulation (21). It is unlike d-amphetamine in that its actions are attenuated by reserpine pretreatment suggesting that it is causing the release of pooled dopamine rather than newly synthesized transmitter (1,2). Further, it appears to exert no effect on either norepinephrine (23) or serotonin metabolism (36) at doses below 2.0 mg/kg. Nisoxetine is a highly selective inhibitor of norepinephrine uptake (41,42). It increases the amount of norepinephrine in the synapse and thus the amount available for binding to the postsynaptic receptors. It has been shown to have little or no effect on dopamine or serotonin, except at doses much higher than needed to block reuptake of norepinephrine (42). Thus, because of amfonelic acid and nisoxetine's high degree of selectivity for dopamine and noradrenergic neurons, respectively, these drugs were used to study the role which dopamine and/or norepinephrine play in mediating the potentiation by d-amphetamine of the threshold lowering effect of morphine.

METHOD

Testing Procedures and Data Analysis

Four male albino F-344 rats (Charles River Laboratories) each weighing approximately 300 grams were used in this experiment. Methods of electrode implantation, training, determination of reward thresholds and histological procedures are the same as those described in the preceding paper (21).

Experiment 1

Animals were injected intraperitoneally with either amfonelic acid or saline, followed immediately by a subcutaneous injection of either morphine or saline. Amfonelic acid was prepared as previously described (21).

Experiment 2

Animals were injected intraperitoneally with either nisoxetine hydrochloride (Lilly Pharmaceuticals Company) or saline, followed 5 minutes later by a subcutaneous injection of either morphine or saline. Nisoxetine hydrochloride was dissolved in isotonic saline.

RESULTS

Experiment 1

Table 1 shows the mean pre-saline threshold in μA plus the post minus pre-saline threshold difference and its standard deviation for each animal. z-Scores after drug treatment were based on these difference scores.

Figure 1 shows the mean (n = 4) effect of 0.063 to 0.50 mg/kg amfonelic acid alone on the reward threshold. All animals showed a significant ($p<0.05$) dose-dependent lowering of the threshold for rewarding brain stimulation following administration of amfonelic acid. Figure 2 shows the effect of morphine alone and in combination with an ineffective dose of amfonelic acid (0.063 mg/kg). Morphine alone lowered the reward threshold and when it was administered concomitantly with amfonelic acid, a greater lowering was seen. The magnitude of this effect always exceeded that observed with either drug alone and was often more than additive. The mean prethreshold in μA for each of the test animals as well as their change score (± SD) is given in the table.

TABLE 1

THE MEAN PRE-SALINE THRESHOLD AND POST MINUS PRE-SALINE THRESHOLD DIFFERENCE ± SD FOR EACH ANIMAL IN μA

Animal	Pre-saline Threshold	Post Minus Pre-saline Difference ± SD*
301	57.9	7.1 ± 4.9*
325	56.0	7.8 ± 5.7
306	25.8	0.3 ± 3.4
304	28.3	5.3 ± 2.7

*z-Scores after drug treatment for each animal are based on these difference scores.

Experiment 2

The mean (n = 4) effect of 1.25 to 20.0 mg/kg of nisoxetine on the reward threshold is shown in Fig. 3. Nisoxetine alone had no significant effect on the threshold. However, it did vary in different animals with two animals showing a lowering and two

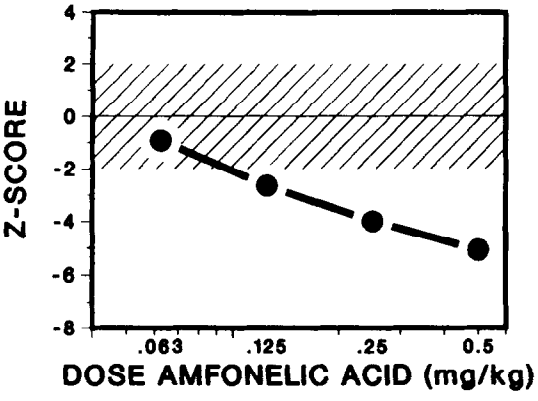

FIG. 1. Mean standard score (z-score) changes in the reward threshold from pre- to post-amfonelic acid for four animals. A z-score of ± 2 (shaded area) indicates the 95% confidence limits.

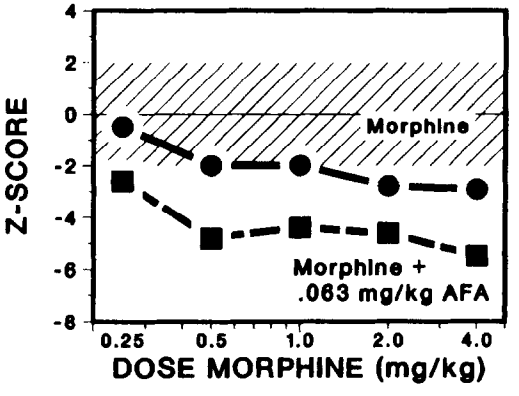

FIG. 2. Mean standard score (z-score) changes in the reward threshold for pre- to postdrug for morphine alone (circles) and in combination with 0.063 mg/kg amfonelic acid (squares) for four animals.

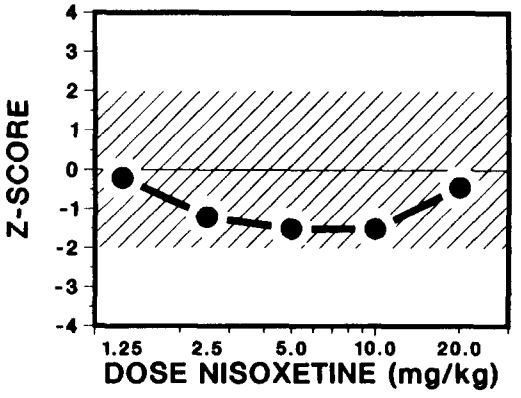

FIG. 3. Mean SEM standard score (z-score) changes in reward threshold from pre- to post-nisoxetine for four animals.

having no effect. Similarly, the effect of nisoxetine on morphine's lowering of the reward threshold was also variable (Fig. 4). In two animals the thresholds following the combination of these drugs were lower than those seen with morphine alone. In one, morphine's effect was not as great at 1.0 mg/kg when administered with nisoxetine, but there seemed to be little or no difference at the other doses of morphine. In one there were no differences between the effect of morphine alone and in combination with nisoxetine.

Histology

Histological examination revealed that the tips of the stimulating electrodes were located in the lateral hypothalamic region of the medial forebrain bundle.

DISCUSSION

As in previous studies (24), morphine lowered the threshold for rewarding brain stimulation to the MFB-LH. Amfonelic acid, when administered by itself, also caused threshold lowerings and these were greater than those seen with morphine alone. When a dose of amfonelic acid which alone caused no significant change in the reward threshold was administered concomitantly with morphine, the threshold lowerings observed were of a greater magnitude than with either drug alone, and were often more than additive.

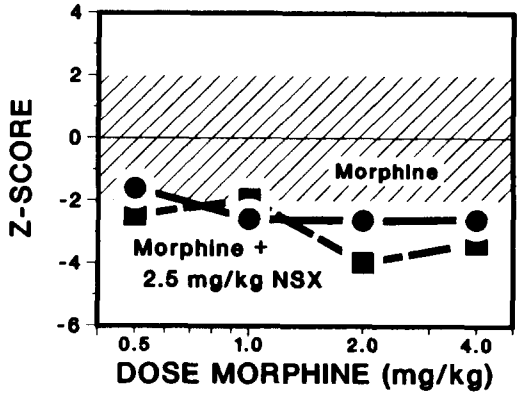

FIG. 4. Mean standard score (z-score) changes in reward threshold from pre- to postdrug for morphine alone (circles) and in combination with 2.5 mg/kg nisoxetine (squares) for four animals.

The results of the effects of amfonelic acid are in accord with previous findings (21), in that they suggest that amfonelic acid had abuse potential. The threshold changes seen are similar to those seen with many drugs of abuse, including stimulants such as cocaine (10) and d-amphetamine (11) and opioids like morphine (24) or heroin (16). Additionally, the magnitude of the threshold lowerings following amfonelic acid suggest that this drug may be even more reinforcing than either morphine or heroin.

Nisoxetine, on the other hand, did not yield consistent results across either animals or drug dose. Alone, it either lowered or had no effect on the reward threshold and when administered together with morphine, nisoxetine either attenuated, potentiated or had no effect on morphine's threshold lowering effect.

These results suport the hypothesis that dopamine plays a critical role in mediating brain-stimulation reward. The finding that a dopamine agonist in combination with morphine produces threshold lowerings greater than those seen with either drug alone suggests an opioid-dopaminergic interaction. The question still exists, however, as to whether facilitation of brain-stimulation reward by drugs of abuse is via activation of endogenous opioid or dopamine containing neurons. The use of naloxone to distinguish between opioid and catecholamine systems has yielded mixed results. It has been reported that naloxone either has no effect on (22,39) or decreases (4,34) response rates for self-stimulation. It has also been shown to have no effect on brain-stimulation reward thresholds following either chronic (26) or acute (11) administration. Studies using dopaminergic drugs, however, have yielded more consistent results. Dopamine antagonists such as haloperidol (9,38), pimozide (5, 38, 40), and chlorpromazine (12) increase threshold or decrease response rates for ICS.

Evidence of opioid modulation of dopamine neurons is also consistent with the idea that brain-stimulation reward is mediated via dopaminergic activity. Reports that there are opiate receptors localized on presynaptic dopamine neurons which appear to play a role in regulating the synthesis and release of dopamine (29–31), and that there is increased turnover of DA following morphine administration (8,33), suggests that the opioids may influence brain-stimulation reward via modulation of dopamine neurons. A number of other studies also provide evidence of opioid modulation of dopaminergic activity. It has been shown that morphine or DALA (a synthetic enkephalin analog) (27) produces an increase in locomotor activity when administered into the ventral tegmental area (7). This is antagonized either by administration of fluphenazine (19) or destruction of dopamine terminals in the nucleus accumbens with 6-OHDA (20). Furthermore, while amfonelic acid, like d-amphetamine, increases the amount of dopamine released per impulse, it also decreases the firing rate of dopaminergic neurons (14). Morphine, on the other hand, increases cell firing rates (25), thus making it likely that the coadministration of these two drugs would result in an even greater release of dopamine into the synapse than would be seen with either drug alone. In summary, it appears that amfonelic acid has abuse potential. These results further suggest that dopamine may play a more critical role than norepinephrine in modulating opiate-induced reinforcement. Based on these findings, it is likely that the potentiation of the lowering of the reward threshold observed following combinations of morphine and d-amphetamine are due to increased dopaminergic and not noradrenergic activity. Thus it seems likely that the reinforcing effects of opioid drugs may be the result of their regulation of mesolimbic dopamine activity, and not due to changes in noradrenergic activity.

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REFERENCES

1. Aceto, M. D.; Botton, I.; Levitt, M.; Martin, R.; Bentley, H. C.; Speight, P. T. Pharmacologic properties and mechanism of action of amfonelic acid. Eur. J. Pharmacol. 10:344–354; 1970.
2. Aceto, M. D.; Harris, L. S.; Lesher, G. Y.; Pearl, J.; Brown, T. G. Pharmacological studies with 7-benzyl-1-ethyl-1,4-dihydro-6-oxo-1,8-naphthyridine-3-carboxylic acide. J. Pharmacol. Exp. Ther. 158: 286–293; 1967.
3. Bain, G. T.; Kornetsky, C. Naloxone attenuation of the effect of cocaine on rewarding brain stimulation. Life Sci. 40:1119–1125; 1987.
4. Belluzzi, J.; Stein, L. Enkephalins may mediate euphoria and drive-reduction reward. Nature 266:556–558; 1977.
5. Bird, M.; Kornetsky, C. The effects of pimozide on stimulus discrimination and rewarding electrical brain stimulation. Soc. Neurosci. Abstr. 11:1190; 1985.
6. Breese, G. R.; Howard, J. L.; Leahy, J. P. Effect of 6-hydroxydopamine on electrical self-stimulation of the brain. Br. J. Pharmacol. 43: 255–257; 1971.
7. Broekkamp, C. L.; Phillips, A. G.; Cools, A. T. Stimulant effects of enkephalin injection into the dopaminergic A10 area. Nature 278: 560–562; 1979.
8. Clouet, D. H.; Ratner, M. Catecholamine biosynthesis in brains of rats treated with morphine. Science 168:854–856; 1970.
9. Esposito, R. U.; Faulkner, W.; Kornetsky, C. Specific modulation of brain stimulation reward by haloperidol. Pharmacol. Biochem. Behav. 10:937–940; 1979.
10. Esposito, R. U.; Motola, A. H. D.; Kornetsky, C. Cocaine: Acute effects on reinforcement thresholds for self-stimulation behavior to the medial forebrain bundle. Pharmacol. Biochem. Behav. 8:437–439; 1978.
11. Esposito, R. U.; Perry, W.; Kornetsky, C. Effects of d-amphetamine and naloxone on brain stimulation reward. Psychopharmacology (Berlin) 69:187–191; 1980.
12. Esposito, R. U.; Perry, W.; Kornetsky, C. Chlorpromazine and brain-stimulation reward: Potentiation of effects by naloxone. Pharmacol. Biochem. Behav. 15:903–905; 1981.
13. German, D. C.; Bowden, D. M. Catecholamine systems as the neural substrate for intracranial self-stimulation: A hypothesis. Brain Res. 73:381–419; 1974.
14. German, D. C.; Harden, H.; Sanghera, M. K.; Mann, D.; Kiser, R. S.; Miller, H. H.; Shore, P. A. Dopaminergic neuronal responses to a non-amphetamine CNS stimulant. J. Neural Transm. 44:39–49; 1979.
15. Gunne, L.-M.; Jonsson, J.; Fuxe, K. Effects of morphine intoxication on brain catecholamine neurons. Eur. J. Pharmacol. 5:338–342; 1969.
16. Hubner, C. B.; Kornetsky, C. The effects of heroin on brain-stimulation reward. Soc. Neurosci. Abstr. 10:1106; 1984.
17. Hubner, C. B.; Bain, G. T.; Kornetsky, C. The combined effect of morphine and d-amphetamine on the threshold for brain-stimulation reward. Pharmacol. Biochem. Behav. 28:311–315; 1987.
18. Jasinski, D. R.; Nutt, J. G. Progress report on the assessment program on the NIMH addiction center. In: Report of the thirty-fourth annual scientific meeting committee on problems of drug dependence. Michigan. NIMH: 1972:442–477.
19. Kalivas, P. W.; Widerlov, E.; Stanley, D.; Breese, G.; Prange, A. J. Enkephalin action on the mesolimbic system: A dopamine-dependent and a dopamine-independent increase in locomotor activity. J. Pharmacol. Exp. Ther. 227:229–237; 1983.
20. Kelley, A. E.; Stinus, L.; Iversen, S. D. Interactions between D-Ala-Met-enkephalin, A10 dopaminergic neurons, and spontaneous behavior in the rat. Behav. Brain Res. 1:3–24; 1980.
21. Knapp, C.; Kornetsky, C. The effects of amfonelic acid alone and in combination with naloxone on brain-stimulation reward. Pharmacol. Biochem. Behav. 32:977–982; 1989.
22. Lorens, S. A.; Sainati, S. M. Naloxone blocks the excitatory effect of ethanol and chlordiazepoxide on lateral hypothalamic self-stimulation behavior. Life Sci. 23:1359–1364; 1978.
23. McMillen, B. A.; Shore, P. A. Amfonelic acid, a non-amphetamine stimulant, has marked effects on brain dopamine metabolism but not noradrenaline metabolism: association with differences in neuronal storage systems. J. Pharm. Pharmacol. 30:464–466; 1978.
24. Marcus, R.; Kornetsky, C. Negative and positive intracranial reinforcement thresholds: Effects of morphine. Psychopharmacologia 38:1–13; 1974.
25. Matthews, R. T.; German, D. C. Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience 11:617–625; 1984.
26. Perry. W.; Esposito, R. U.; Kornetsky, C. Effects of chronic naloxone treatment on brain-stimulation reward. Pharmacol. Biochem. Behav. 14:247–249; 1981.
27. Pert, C. B.; Pert, A.; Chang, J.-K.; Fong, B. T. W. (D-Ala)-Met-enkephalinamide: A potent, long-lasting synthetic pentapeptide analgesic. Science 194:330–332; 1976.
28. Pettit, H. O.; Ettenberg, A.; Bloom, F. E.; Koob, G. F. Destruction of dopamine in nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berlin) 84:167–173; 1984.
29. Pollard, H.; Llorens-Cortes, C.; Schwartz, J. C. Enkephalin receptors on dopaminergic neurons in rat striatum. Nature 268:745–747; 1977.
30. Pollard, H.; Llorens, C.; Schwartz, J. C. Opiate receptors on mesolimbic dopaminergic neurons. Neurosci. Lett. 7:295–299; 1977.
31. Schwartz, J. C.; Pollard, H.; Llorens, C.; Malfroy, B.; Gros, C.; Pradelles, P.; Dray, F. Endorphins and endorphin receptors in striatum: Relationships with dopaminergic neurons. Adv. Biochem. Psychopharmacol. 18:245–264; 1978.
32. Shore, P. A. Actions of amfonelic acid and other non-amphetamine stimulants on the dopamine neuron. J. Pharm. Pharmacol. 28: 855–857; 1976.
33. Smith, J. E.; Co, C.; Freeman, M. E.; Sands, M. P.; Lane, J. D. Neurotransmitter turnover in rat striatum is correlated with morphine self-administration. Nature 287:152–153; 1980.
34. Stein, L. The chemistry of reward. In: Routtenberg, A., ed. Biology of reinforcement: Facts of brain-stimulation reward. New York: Academic Press; 1980:109–130.
35. Stein, L.; Ray, O. S. Brain stimulation reward "thresholds" self-determined in rat. Psychopharmacologia 1:251–256; 1960.
36. Waldmeier, P. C.; Buchle, A. M.; Stoecklin, K.; Fehr, B.; Feldtrauer, J.-J. The effects of amfonelic acid on 5-HT metabolism in rat brain. J. Neural Transm. 57:149–165; 1983.
37. Wauquier, A. The influence of psychoactive drugs on brain self-stimulation in rats: A review. In: Wauquier, A.; Rolls, E. T., eds. Brain-stimulation reward. Amsterdam: North Holland; 1976:123–170.
38. Wauquier, A.; Niemegeers, C. J. E. Intracranial self-stimulation in rats as a function of various stimulus parameters. II. Influence of haloperidol, pimozide and pipamperone on medial forebrain stimulation with monopolar electrodes. Psychopharmacologia 27:191–202; 1972.
39. Wauquier, A.; Niemegeers, C. J. E.; Lal, H. Differential antagonism by naloxone of inhibitory effects of haloperidol and morphine on brain self-stimulation. Psychopharmacologia 37:303–310; 1974.
40. Wise, R. A. Neuroleptic attenuation of intracranial self-stimulation: reward or performance deficits? Life Sci. 22:535–542; 1978.
41. Wong, D. T.; Bymaster, F. P. The comparison of fluoxetine and nisoxetine with tricyclic antidepressants in blocking the neurotoxicity of p-chloroamphetamine and 6-hydroxydopamine in the rat brain. Res. Commun. Chem. Pathol. Pharmacol. 15:221–231; 1976.
42. Wong, D. T.; Bymaster, F. P. Effect of nisoxetine on uptake of catecholamines in synaptosomes isolated from discrete regions of rat brain. Biochem. Pharmacol. 25:1979–1983; 1976.