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Central Monoaminergic Changes Induced by Morphine in Hypoalgesic and Hyperalgesic Strains of Domestic Fowl

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SUFKA, K. J., D. A. HOGANSON AND R. A. HUGHES. *Central monoaminergic changes induced by morphine in hypoalgesic and hyperalgesic strains of domestic fowl.* PHARMACOL BIOCHEM BEHAV **42**(4) 781-785, 1992.—The present research examined morphine dose-response effects on both the formalin test and on CNS monoamine (MA) levels and the metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) in hypoalgesic (76) and hyperalgesic (SN) strains of domestic fowl. Morphine produced a significant hypoalgesic response in the 76 strain at 15–45 mg/kg and a significant hyperalgesic response in the SN strain at 5–10 mg/kg. In subsequent experiments, analyses of whole brain (minus tectum), brainstem, and spinal cord MA, DOPAC, and 5-HIAA via high-performance liquid chromotagraphy with electrochemical detection (HPLC-EC) were performed following morphine administration in both the 76 and SN strains. Morphine produced a significant elevation of brain dopamine (DA) and a significant elevation of brain, brainstem, and spinal cord serotonin (5-HT) in both the 76 and SN strains. Morphine elevated brain norepinephrine (NE) in the 76 strain. However, morphine failed to affect brain NE in the SN strain. This distinct morphine effect on brain NE differentiates strain-dependent hypoalgesia and hyperalgesia in domestic fowl.

Opioids Opiates Morphine Monoamines Dopamine Norepinephrine Serotonin
Formalin test Nociception Hypoalgesia Hyperalgesia Domestic fowl

THE contribution of CNS monoamines (MAs), principally serotonin (5-HT) and norepinephrine (NE), in the expression of morphine hypoalgesia is well documented [for a review, see (6,7)]. For example, 5-HT synthesis inhibitors (20), 5-HT receptor antagonists (3,4,21), and NE antagonists (12) reduced the antinociceptive effects of morphine. In addition, administration of morphine has been shown to elevate 5-HT and NE levels in the CNS (1,2,14). Further support for the involvement of these MAs in the modulation of nociception is provided by the observation that administration of 5-HT and NE receptor agonists produce hypoalgesic effects (13).

Although morphine typically produces hypoalgesic effects in most species, research in this laboratory has identified a biological model in which morphine produces a hyperalgesic response. This unusual morphine effect is strain dependent (9), naloxone reversible (8–10), primarily mediated by μ-opioid receptors (18), displays the dose and temporal characteristics typical of morphine hypoalgesia (17), and has been observed in both thermal and chemoinflammatory nociceptive tests (8,10). Although these studies more fully characterize hyperalgesic effects in fowl, the neuropharmacologic substrates of morphine hyperalgesia are unclear. Given the documented role of MA functioning in morphine hypoalgesic effects, a comparison of MA activity in hypo- and hyperalgesic strains of fowl may elucidate possible mediators of atypical hyperalgesia in fowl. The present research, therefore, examined levels of CNS MA and the metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) via high-performance liquid chromatography with electrochemical detection (HPLC-EC) following morphine administration in both hyperalgesic and hypoalgesic strains of domestic fowl.

EXPERIMENT 1

This first experiment sought to replicate strain-dependent morphine hypoalgesic and hyperalgesic effects (9) in domestic fowl on a modified version of the formalin test (5,10). Although we were no longer able to obtain the same strains of fowl used in our previous work (9), we were able to locate both a hypoalgesic (76) and hyperalgesic (SN) strain from an alternative supplier (Hy-Line International, Dallas Center, IA). Ex-

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amination of morphine effects on the formalin test in these two strains of fowl were performed in separate experiments.

METHOD

Subjects

Chicks (SN or 76; Hy-Line International) were obtained several hours after hatch and housed in pairs, under 24-h overhead fluorescent illumination, in chambers that provided physical separation but not auditory or visual isolation [see (18) for complete housing apparatus description]. Chicks were permitted free access to food (Wayne pullet starter) and tap-water. Room temperature was maintained at 32.0°C for the first week and 29.0°C thereafter.

Procedure

At 14–15 days posthatch, chicks received IM injections of morphine or saline in a volume of 1.0 ml/kg and were returned to their home cages. The morphine dose administered to the 76-hypoalgesic strain was 15.0, 30.0, or 45.0 mg/kg (n = 10). Pilot work demonstrated that lower doses of morphine did not produce either significant hypo- or hyperalgesic effects in the 76 strain. The morphine dose administered to the SN-hyperalgesic strain was 5.0 or 10.0 mg/kg (n = 10). Thirty minutes after IM injections, chicks were transported to an adjacent room where formalin tests were conducted. For the formalin test, chicks received an intraplantar (IPL) injection of 0.15% formalin in a total volume of 50 μl and were placed into modified LVE sound-attenuating chambers [see (10) for complete procedural details and test apparatus description]. During a 2-min observation period, the number of foot-lifts were recorded by trained observers. Each animal was tested individually and returned to its home cage after the nociceptive test.

Data were analyzed using one-way analysis of variance (ANOVA) and one-tailed power-adjusted Student's t-tests (11). Significance was considered at $p < 0.05$.

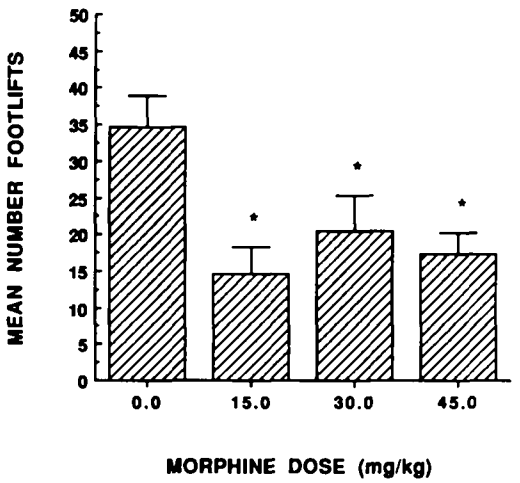

FIG. 1. Effects of morphine on chemoinflammatory nociception in the 76 strain. Bars represent mean number of foot-lifts following IPL administration of 50 μl 0.15% formalin solution during a 2-min observation period (vertical lines = SEM). *Significant morphine hypoalgesic effects.

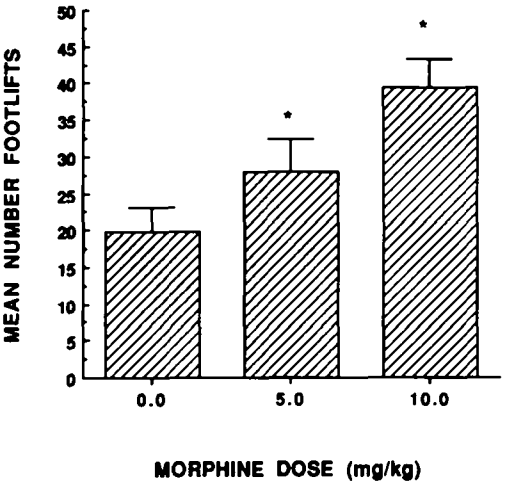

FIG. 2. Effects of morphine on chemoinflammatory nociception in the SN strain. Bars represent mean number of foot-lifts following IPL administration of 50 μl 0.15% formalin solution during a 2-min observation period (vertical lines = SEM). *Significant morphine hyperalgesic effects.

RESULTS AND DISCUSSION

The results of the formalin tests for the 76 strain are summarized in Fig. 1. Chicks that received morphine exhibited fewer foot-lift responses compared to saline-treated chicks. A one-way ANOVA of these data revealed a significant morphine treatment effect, $F(3, 36) = 5.18$, $p < 0.005$. Further analyses demonstrated a significant decrease in mean foot-lifts (i.e., hypoalgesic response) at each morphine dose (15, 30, and 45 mg/kg) compared to the mean foot-lift score of saline-treated chicks, $t(39) = 3.63$, 2.57, and 3.11, $p' < 0.01$.

The results of the formalin test for the SN strain are summarized in Fig. 2. Chicks that received morphine displayed an increase in foot-lift responding compared to saline-treated chicks. A one-way ANOVA revealed a significant morphine treatment effect, $F(2, 27) = 6.36$, $p < 0.006$. Further analyses demonstrated a significant increase in mean foot-lifts (i.e., hyperalgesic response) at both the 5.0- and 10.0-mg/kg morphine doses compared to the mean foot-lift score of saline-treated chicks, $t(29) = 2.07$ and 3.58, $p' < 0.03$.

The results of this first experiment demonstrate strain-dependent morphine hypoalgesic (76 strain) and hyperalgesic (SN strain) effects on the formalin test. These results are consistent with earlier reports of strain-dependent morphine hypoalgesia and hyperalgesia on thermal nociception in strains from our previous animal supplier (9).

EXPERIMENT 2

This second set of studies examined CNS MA, DOPAC, and 5-HIAA levels following morphine administration in the 76 (hypoalgesic) and SN (hyperalgesic) strains via HPLC-EC. Examination of morphine effects on CNS MA, DOPAC, and 5-HIAA in the 76 and SN strains was performed in separate experiments.

METHOD

Subject and housing characteristics were as described in Experiment 1. At 14–15 days posthatch, chicks received IM

injections of morphine (76 strain: 15 and 30 mg/kg, $n = 10$; SN strain: 5 and 10 mg/kg, $n = 8$) or saline in a volume of 1.0 ml/kg and were returned to their home cage. Thirty minutes after injection, animals were killed via rapid decapitation and whole brain (minus tectum), brainstem, and spinal cord were dissected on dry ice. Tissues were weighed to the nearest 0.1 mg and were placed into separate 1.5-ml polyethylene tubes and stored at −70°C until use. HPLC-EC of CNS MA was performed according to methods described by Saller and Salama (15). Frozen tissues (25–1,000 mg) were placed into a sonication cuvette with 38 vol homogenization buffer that consisted of 7 vol 0.1 M monobasic sodium phosphate (adjusted to pH 4.0 using a saturated citric acid solution) containing 1 mM disodium EDTA and 1 mM sodium octanesulfonic acid and 3 vol acetonitrile. Two additional volumes of the buffer containing the internal standard, *N*-methyl 5 hydroxytryptamine, (250 ng/ml) were added to each sample before sonication. The samples were homogenized using a W-185 sonicator (Heat Systems-Ultrasonics Inc., Plainview, NY). Portions of the samples were placed into 15-ml glass centrifuge tubes and centrifuged at 15,000 × *g* for 20 min at 4°C. Portions of the extracts were removed from the pellets and frozen at −70°C until approximately 12 h before being assayed. At this time, samples were thawed and left at room temperature for 12 h. Each sample was then recentrifuged before the HPLC assay. The room temperature incubation was found to be necessary to allow for decay of compounds observed in the solvent front that interfered with the assay of norepinephrine.

Portions of the tissue extracts were injected manually using a Beckman 210A injector (Beckman Instruments, Inc., Berkeley, CA) equipped with a 20-μl loop. An Altex 5-μm, 0.46 × 25 cm C_{18} Ultrasphere reverse-phase column (Beckman Instruments, Inc.) was used. The analytical column was protected from sample contaminants by using a 0.46 × 4.5 cm guard column between the injector and analytical column.

The mobile phase consisted of 100 vol 0.1 M monobasic sodium phosphate containing 1 mM disodium EDTA and 1 mM sodium octanesulfonic acid (adjusted to pH 4.0 with a saturated solution of citric acid) and 10.5 vol acetonitrile. The mobile phase was filtered through a 0.45-μm nylon filter and degassed under vacuum. The flow rate of the mobile phase was maintained at 1 ml/min with a Beckman Model 110B pump. The column effluent was passed through the flow cell of a Bio-Rad Model 1340 electrochemical detector (Bio-Rad Laboratories, Hercules, CA). The electrical potential applied to the working electrode was 0.65 V. Peaks were integrated using a Hewlett-Packard Model 3392A integrator (Hewlett-Packard Co., Avondale, PA).

Data were analyzed using one-way ANOVA and one-tailed power-adjusted Student's *t*-test (11). Significance was considered at $p < 0.05$.

TABLE 1

EFFECTS OF SYSTEMIC MORPHINE ON CNS MONOAMINE AND METABOLITE LEVELS IN THE 76 (HYPOALGESIC) STRAIN

CNS Monoamines	Morphine Dose (mg/kg) 0.0	15.0	30.0
Brain			
NE	307.5 (15.5)	392.1* (44.1)	356.7 (16.1)
DOPAC	72.3 (6.7)	111.1* (24.5)	74.8 (7.7)
DA	681.9 (27.3)	911.9* (55.3)	761.9 (51.4)
5-HIAA	152.7 (20.1)	121.0 (12.6)	163.6 (32.1)
5-HT	1,314.3 (30.5)	1,648.9* (51.7)	1,662.4* (67.9)
Brainstem			
NE	907.1 (81.8)	953.4 (52.8)	1,029.3 (48.5)
DOPAC	102.4 (41.0)	80.0 (8.8)	76.0 (10.2)
DA	326.8 (40.8)	331.9 (18.5)	334.2 (13.7)
5-HIAA	332.7 (64.5)	286.6 (38.6)	291.1 (43.9)
5-HT	1,464.1 (94.4)	1,806.7* (75.1)	1,901.0* (57.8)
Spinal cord			
NE	256.3 (10.2)	225.2 (14.4)	247.5 (15.3)
DA	138.3 (14.1)	109.4 (9.9)	108.5 (9.3)
5-HIAA	267.3 (77.4)	210.5 (34.1)	178.9 (18.2)
5-HT	886.7 (50.3)	1,010.4 (52.1)	1,085.9* (61.3)

Values are expressed as mean ng/g wet weight tissue for 10 determinations. Numbers in parentheses are SEM.

*Significant MA or metabolite elevation compared to saline control, $p < 0.05$.

TABLE 2

EFFECTS OF SYSTEMIC MORPHINE ON CNS MONOAMINE AND METABOLITE LEVELS IN THE SN (HYPERALGESIC) STRAIN

CNS Monoamines	Morphine Dose (mg/kg) 0.0	5.0	10.0
Brain			
NE	335.1 (12.3)	324.8 (12.1)	339.4 (12.2)
DOPAC	42.2 (4.3)	54.0 (3.3)	57.5* (4.6)
DA	754.0 (27.9)	777.0 (22.5)	832.9* (39.2)
5-HIAA	156.5 (15.3)	141.8 (12.3)	159.9 (6.0)
5-HT	1,520 (31.3)	1,637.0 (19.9)	1,764.2* (61.0)
Brainstem			
NE	976.2 (33.6)	1014.6 (35.4)	995.9 (53.8)
DOPAC	40.5 (4.2)	50.6 (7.0)	41.8 (4.2)
DA	313.0 (11.9)	320.8 (9.4)	304.4 (15.1)
5-HIAA	305.5 (18.8)	283.9 (12.6)	291.5 (15.5)
5-HT	1,831.6 (69.9)	2,003.0 (39.4)	2,029.1* (77.1)
Spinal cord			
NE	230.3 (8.0)	212.0 (18.1)	261.4 (18.9)
DA	105.4 (19.8)	73.2 (5.1)	86.4 (11.4)
5-HIAA	207.0 (14.2)	269.6* (18.4)	223.6 (21.2)
5-HT	851.8 (43.3)	1,008.4 (105.5)	1,127.0* (77.4)

Values are expressed as mean ng/g wet weight tissue for eight determinations. Numbers in parentheses are SEM.

*Significant MA or metabolite elevation as compared to saline control, $p < 0.05$.

RESULTS AND DISCUSSION

The results of the HPLC-EC studies for the 76 and SN strains are summarized in Tables 1 and 2, respectively. For the 76 strain (see Table 1), morphine produced a significant increase in brain NE, $t(28) = 2.09$, DOPAC, $t(28) = 1.79$, $p^s < 0.05$, and DA, $t(28) = 3.50$, $p < 0.001$, at the 15-mg/kg dose and a significant increase in 5-HT at the 15- and 30-mg/kg doses, $t(28) = 4.52$ and 4.70, respectively, $p^s < 0.001$. In this 76 strain, morphine produced a significant increase in brainstem 5-HT at the 15- and 30-mg/kg morphine doses, $t(28) = 3.13$ and 4.00, respectively, $p^s < 0.001$, and a

TABLE 3

SUMMARY OF SIGNIFICANT DIRECTIONAL CHANGES OF MA-INDUCED BY MORPHINE IN 76 (HYOPALGESIC) AND SN (HYPERALGESIC) STRAINS OF DOMESTIC FOWL

	Strain	
CNS Monoamines	76	SN
Brain		
NE	↑	–
DA	↑	↑
5-HT	↑	↑
Brainstem		
5-HT	↑	↑
Spinal cord		
5-HT	↑	↑

significant increase in the spinal cord 5-HT at the 30-mg/kg dose, $t(28) = 2.57, p < 0.01$.

In the SN strain (see Table 2), morphine produced a significant increase in brain DOPAC, $t(23) = 2.64, p < 0.01$, DA, $t(23) = 1.82, p < 0.05$, and 5-HT, $t(23) = 4.19, p < 0.001$, at the 10-mg/kg dose. Morphine also produced a significant increase in brainstem and spinal cord 5-HT, $t(23) = 2.17$ and 2.45, respectively, $p^s < 0.05$, at the 10-mg/kg dose. As well, morphine produced a significant increase in spinal cord 5-HIAA, $t(23) = 2.44, p < 0.05$, at the 5-mg/kg dose.

To permit strain comparisons of morphine effects on CNS MA in this second experiment, a summary of the significant HPLC-ED data is provided in Table 3. Because changes in CNS MA metabolites presumably reflect changes in MA activity, we chose to summarize only the MA data. Both the 76 and SN strains exhibited similar changes in CNS DA and 5-HT following morphine administration. Morphine elevated brain DA levels and elevated brain, brainstem, and spinal cord 5-HT levels. Morphine produced a significant increase in brain NE in the 76 strain. However, the SN strain failed to show such NE response to morphine.

The effects of morphine on CNS 5-HT in the 76 and SN strains of fowl are consistent with observations that morphine elicits an increase in CNS 5-HT in rats (1,2,7). Morphine administration also increased brain DA activity in both strains of fowl. This morphine effect is consistent with reports that morphine increases DA turnover rates in other animal models (16,19). Morphine administration has been shown to elevate CNS NE in mammals (14). In the present research, morphine-induced increases in brain NE were only detected in the 76 (hypoalgesic) strain. The lack of an increase in NE in the SN (hyperalgesic) strain may account for the atypical morphine effects observed in this strain.

GENERAL DISCUSSION

In most animals, hypoalgesia is a typical behavioral outcome following morphine administration. However, morphine has been shown to produce strain-dependent hyperalgesic effects as well (9). Previous attempts to identify factors that differentiate morphine hypoalgesia and hyperalgesia have shown that hyperalgesia is much like typical morphine hypoalgesia. For example, morphine hyperalgesia a) exhibits the dose and temporal characteristics typical of morphine hypoalgesia (17), b) is primarily mediated by μ-receptors (18), and c) is not a unique outcome of thermal nociceptive tests (8,10). The vendor that supplied the original hypoalgesic and hyperalgesic strains no longer breeds those animals. The present research (Experiment 1) thereby documents strain-dependent morphine hypoalgesic and hyperalgesic effects in two new strains of fowl from another animal supplier. Thus, strain-dependent hypoalgesia and hyperalgesia is not restricted to breeds from a single supplier. It may be significant in terms of genetic factors that the hypoalgesic breed from both suppliers is a heavy-body brown egg strain and the hyperalgesic breed is a light-body white egg strain.

In the present research (Experiment 2), morphine produced several similar changes (e.g., brain 5-HT and DA) in CNS MA levels in these two strains of fowl. However, one distinct difference was noted between the 76 and SN strains: The 76 strain exhibited an increase in brain NE while the SN strain failed to display such an NE response. This morphine effect on brain NE differentiates strain-dependent hypoalgesia and hyperalgesia in domestic fowl. Whether the lack of an NE response to morphine subserves atypical hyperalgesia remains to be determined. This laboratory is currently investigating that possibility.

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REFERENCES

1. Aiello-Malmberg, P.; Bartolini, A.; Bartolini, R.; Galli, A. Effects of morphine, physostigmine and raphe nuclei stimulation on 5-hydroxytryptamine release from the cerebral cortex of the cat. Br. J. Pharmacol. 65:547-555; 1979.
2. Bardon, T.; Ruckebusch, M. Changes in 5-HIAA and 5-HT levels in lumbar CSF following morphine administration to conscious dogs. Neurosci. Lett. 49:147-151; 1984.
3. Crisp, T.; Smith, D. J. A local serotonergic component involved in the spinal antinociceptive action of morphine. Neuropharmacology 28:1047-1053; 1989.
4. Crisp, T.; Stafinsky, J. L.; Uram, M.; Perni, V. C.; Weaver, M. F.; Spanos, L. J. Serotonin contributes to the spinal antinociceptive effects of morphine. Pharmacol. Biochem. Behav. 39:591-595; 1991.
5. Dubuisson, D.; Dennis, S. G. The formalin test: A quantitative study of the analgesic effects of morphine, meperidine, and brainstem stimulation in rats and cats. Pain 4:161-164; 1977.
6. Fenk, H.; Cannon, J. T.; Lewis, J. W.; Liebeskind, J. C. Neural and neurochemical mechanisms of pain inhibition. In: Sternback, R. A., ed. The psychology of pain. New York: Raven Press; 1988:25-47.
7. Hamon, M.; Bourgoin, S.; Le Bars, D.; Cesselin, F. *In vivo* and *in vitro* release of central neurotransmitters in relation to pain and analgesia. In: Fields, H. L.; Besson, J. M., eds. Progress in brain research. vol. 77. New York: Elsevier, 1988:431-444.
8. Hughes, R. A. Codeine analgesic and morphine hyperalgesic effects on thermal nociception in domestic fowl. Pharmacol. Biochem. Behav. 35:567-570; 1990.
9. Hughes, R. A. Strain-dependent morphine-induced analgesic and hyperalgesic effects on thermal nociception in domestic fowl. Behav. Neurosci. 104:619-624; 1990.
10. Hughes, R. A.; Sufka, K. J. Morphine hyperalgesic effects on the formalin test in domestic fowl. Pharmacol. Biochem. Behav. 38:247-251; 1991.

11. Kirk, R. E. Experimental design: Procedures for the behavioral sciences. Monterey, CA: Brooks/Cole; 1982.
12. Kuraishi, Y.; Harada, Y.; Satoh, M.; Tagaki, H. Antagonism by phenoxybenzamine of the analgesic effect of morphine injected into the nucleus reticularis gigantocellularis of the rat. Neuropharmacology 18:107–110; 1979.
13. Kuraishi, Y.; Hirota, N.; Satoh, M.; Tagaki, H. Antinociceptive effects of intrathecal opioids, noradrenaline and serotonin in rats: Mechanical and thermal algesic tests. Brain Res. 326:168–171; 1985.
14. Roffman, M.; Cassens, G.; Schildkraut, J. J. The effects of acute and chronic administration of morphine on norepinephrine turnover in rat brain regions. Biochem. Pharmacol. 26:2355–2358; 1977.
15. Saller, C. F.; Salama, A. I. Rapid automated analysis of biogenic amines and their metabolites using reversed-phase high-performance liquid chromatography with electrochemical detection. J. Chromotog. 309:287–298; 1984.
16. Smith, C. B.; Villarreal, J. E.; Bednarczyk, J. H.; Sheldon, M. I. Tolerance to morphine-induced increases in [^{14}C]catecholamine synthesis in mouse brain. Science 170:1106–1108; 1970.
17. Sufka, K. J.; Hughes, R. A. Dose and temporal characteristics of morphine-induced hyperalgesia in domestic fowl. Physiol. Behav. 47:385–387; 1990.
18. Sufka, K. J.; Hughes, R. A.; Giordano, J. Effects of selective opiate antagonists on morphine-induced hyperalgesia in domestic fowl. Pharmacol. Biochem. Behav. 38:49–54; 1991.
19. Sugrue, M. F. Effects of morphine and pentazocine on the turnover of noradrenaline and dopamine in various regions of the rat brain. Br. J. Pharmacol. 5:389; 1974.
20. Tenen, S. S. Antagonism of the analgesic effect of morphine and other drugs by *p*-chlorophenylalanine, a serotonin depleter. Psychopharmacology (Berl.) 12:278–285; 1968.
21. Yaksh, T. L.; Du Chateau, J. C.; Rudy, T. A. Antagonism by methysergide and cinanserin of the antinociceptive action of morphine administered into the periaqueductal gray. Brain Res. 104: 367–372; 1976.