Pharmacology Biochemistry & Behavior, Vol. 17, pp. 419–423, 1982. Printed in the U.S.A.

Morphine Enhances High-Affinity Choline Uptake in Mouse Striatum[1]

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Received 18 February 1982

VALLANO, M. L., M. T. SPOERLEIN AND C. VANDERWENDE. *Morphine enhances high-affinity choline uptake in mouse striatum.* PHARMAC. BIOCHEM. BEHAV. 17(3) 419–423, 1982.—The effects of morphine (2 mg/kg–60 mg/kg) on cholinergic neuronal activity were examined by the method of high-affinity, Na^+-dependent [^{3}H]choline uptake into synaptosomes isolated from mouse corpus striatum. Acute administration of analgesic doses of morphine (10 mg/kg, 20 mg/kg) significantly stimulated choline uptake into synaptosomes in a naloxone-reversible manner. When synaptosomes were directly exposed to pharmacologically effective concentrations of morphine (0.1 μM–10.0 μM) *in vitro* however, choline transport was not signifcantly different from control transport, suggesting that morphine (10 mg/kg, 20 mg/kg) does not stimulate choline uptake by a direct effect on the cholinergic nerve terminal. The possibility that acute morphine administration indirectly enhances striatal cholinergic neuronal activity by inhibiting dopaminergic function was supported pharmacologically since the dopaminergic agonists, apomorphine (10 mg/kg) or amantadine (50 mg/kg), reversed the stimulatory effect of morphine on choline uptake. High-affinity choline transport into synaptosomes was not significantly different from control uptake in response to a sub-analgesic dose of morphine (2 mg/kg) or in response to 60 mg/kg, a dose that elicited hypermotility. These data suggest that analgesic doses of morphine may indirectly enhance cholinergic neuronal activity in the mouse corpus striatum.

Morphine Acetylcholine Dopamine Corpus striatum Mouse Analgesia

THE complex relationship between the behavioral and neurochemical effects of opiates on the central nervous system remains an area of active investigation. The mammalian corpus striatum contains a dense network of opiate receptors [16,22] and the highest concentrations of methionine and leucine enkephalin in the brain [21,31]. Approximately one-third of the enkephalinergic neurons in the striatum terminate presynaptically upon nigrostriatal dopaminergic axons, whereas the remaining receptors may lie on intrastriatal GABAergic and cholinergic neurons [2, 13, 17]. In order to determine the nature of opiate action in the striatum, investigators have attempted to characterize narcotic effects upon individual neurotransmitter systems.

In the mouse, relatively large doses of narcotics elicit a hypermotility response that is dose-dependent and antagonized by pretreatment with naloxone [5,11]. An increase in striatal dopamine release and adenosine 3′, 5′-monophosphate levels were observed in mice that exhibited the hypermotility response [27]. The increase in dopaminergic neuronal transmission was accompanied by a decrease in striatal acetylcholine release [18]. A wide variety of dopaminergic agonists facilitate opiate-induced locomotion, whereas dopaminergic antagonists [9,29] and anticholinesterases [20] diminish the response. These studies suggest that an increase in striatal dopaminergic function and a reduction in cholinergic neurotransmission are related to opiate-induced hypermotility in the mouse.

In contrast, the analgesic response that is elicited by lower doses of morphine is augmented by pretreatment with dopaminergic antagonists and reduced by pretreatment with dopaminergic agonists [28]. Since the mechanisms underlying narcotic-induced analgesia and hypermotility appear to be distinct [14], they may involve opposite effects on the cholinergic and dopaminergic systems depending upon the dosage.

In this report, the effects of various doses of morphine on striatal high-affinity Na^+-dependent choline uptake (HANDU) have been examined in a strain of mice that has been characterized behaviorally with respect to the actions of morphine [24, 28, 29, 30]. In addition, the interactions between striatal cholinergic and dopaminergic systems have been investigated pharmacologically.

METHOD

Male Carworth CF-1 albino mice (20–30 g) were utilized in the experiments. The animals were maintained in a 12 hr dark/light cycle and were used for experimentation between 9:00–10:00 a.m. Temperature remained constant at 24°C.

[1]Supported in part by NIDA Training Grant #5-F31 DA05056 from the National Institutes of Health.
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Purina laboratory chow and water were available ad lib.

Amantadine HCl and dl-β-3,4-dihydroxyphenylalanine (dl-Dopa) were purchased from Sigma. Apomorphine HCl, atropine SO_4 and morphine SO_4 were obtained from Merck. [^{3}H]choline, protosol and aquasol were purchased from New England Nuclear. In addition, the following compounds were donated by the manufacturers: haloperidol (McNeil labs); naloxone HCl (Endo labs). All other compounds were reagent grade and were obtained from commercial sources.

Test compounds or vehicle were administered by the intraperitoneal mode in a volume ranging betwen 0.2–0.3 ml. Doses of basic compounds refer to the salts. Animals were sacrificed 40 min after morphine injection, when peak brain concentrations are obtained [15].

In a separate set of experiments, the direct effect of morphine on choline uptake into synaptosomes was examined by adding morphine or vehicle directly to the sample tube prior to incubation with 0.26 μM choline (0.4 μCi [^{3}H]choline).

The measurement of high-affinity Na^+-dependent [^{3}H]choline uptake into striatal synaptosomes was used as a qualitative index of cholinergic neuronal activity *in vivo* [23]. Choline uptake into striatal synaptosomes was linear over the incubation times and choline concentrations utilized in the experiments. Moreover, when mitochondria and synaptosomes were separated by high-speed centrifugation of the P_2 homogenate over a discontinuous sucrose density gradient [6], HANDU occurred exclusively in the synaptosomal fraction with a negligible contribution by mitochondria (unpublished observations). Subsequent to decapitation, both striata from each brain were rapidly excised, homogenized in 2.0 ml of 0.32 M sucrose and centrifuged at 1000 × g for 10 min. The supernatant was transferred to a clean tube and centrifuged at 17000 × g for 15 min in order to obtain a crude synaptosomal pellet (Whittaker's P_2 pellet). The P_2 pellet was gently resuspended in 1.5 ml of 0.32 M sucrose. All preparative procedures were performed at 4°C. Choline uptake was initiated by adding 100 μl of synaptosomal homogenate to 900 μl of a modified Krebs-Ringer buffer (pH 7.4, prewarmed to 31°C) containing 0.26 μM choline (0.4 μCi [^{3}H]choline). Triplicate samples were incubated for 3 min at 31°C in buffer containing sodium (126.0 mM NaCl; 4.8 mM KCl; 1.3 mM $CaCl_2$; 15.8 mM $NaHPO_4$; 1.4 mM $MgCl_2$; and 2.0 mg/ml dextrose) or buffer containing no sodium (NaCl and Na_2HPO_4 were replaced by 252.0 mM sucrose and 15.8 mM Tris-phosphate). The uptake reaction was terminated by transferring the samples to an ice-bath followed by centrifugation at 6000 × g for 20 min. The synaptosomal pellets containing [^{3}H]choline were surface washed with 2 1-ml aliquots of 0.9% NaCl and solubilized in 1 ml of protosol. The solubilized mixture was placed into a scintillation vial and each sample tube was rinsed with 2 5-ml aliquots of aquasol scintillation cocktail prior to addition of the aquasol to vials. Each sample was neutralized with 0.5 ml of 20% acetic acid and the radioactivity was counted by liquid scintillation spectrometry at 40% efficiency. Protein was determined spectrophotometrically as described by [3]. Na^+-dependent uptake was calculated as the difference between total uptake into synaptosomes that were incubated in Na^+ buffer, and non-specific low-affinity uptake which persists in the Na^+-free buffer. The low-affinity choline transport into striatal synaptosomes represented ≈20% of the total choline transport. The data, pmol choline/3 min/mg protein, are expressed as percentages compared to 100% control uptake. Statistical analysis of paired samples was performed using the Wilcoxon two-tailed signed rank test.

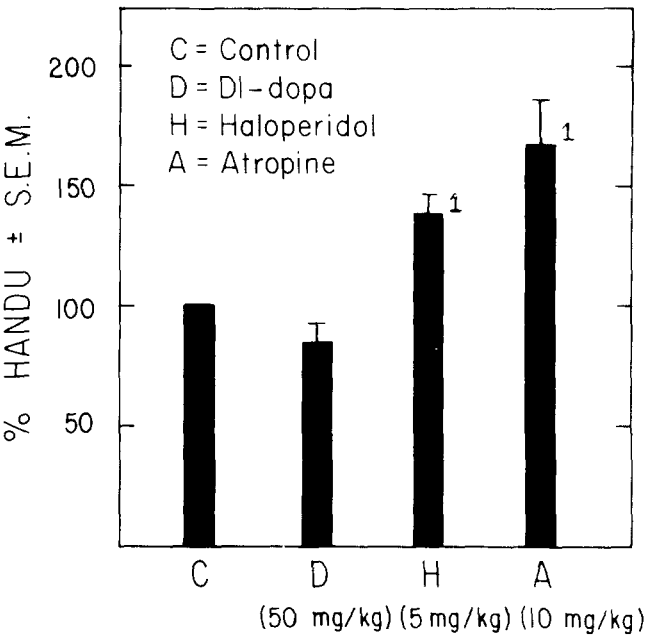

FIG. 1. Effects of pharmacological agents on HANDU into striatal synaptosomes. $^1p=0.03$. dl-Dopa and haloperidol were administered IP 60 min prior to sacrifice. Atropine was administered IP 30 min prior to sacrifice. The data represent Mean ± S.E.M. of at least 4 separate determinations (n for control group = n for experimental group).

RESULTS

In order to substantiate the validity of using HANDU as an index of cholinergic neuronal activity, three compounds whose actions on striatal ACh release have been established were examined for their effects on HANDU into striatal synaptosomes. As shown in Fig. 1, a 31% increase in choline uptake was observed in response to the administration of haloperidol (5 mg/kg), a neuroleptic which blocks postsynaptic dopamine receptors. Atropine (10 mg/kg), a muscarinic cholinergic receptor anatagonist, produced a 57% increase in choline transport. Only a modest reduction in HANDU was observed in response to dl-Dopa (50 mg/kg), a dopaminergic agonist. These data indicate that pharmacological agents which enhance striatal cholinergic neuronal activity also enhance HANDU into mouse striatal synaptosomes.

The effects of morphine (2 mg/kg–60 mg/kg) and naloxone (1 mg/kg) on HANDU into striatal synaptosomes are shown in Fig. 2. A significant increase in HANDU was observed in mice that had received analgesic doses of morphine (10 mg/kg, 20 mg/kg). Pretreatment with naloxone reversed the stimulatory effect of morphine on high-affinity choline transport. When the dosage was increased to 60 mg/kg, morphine elicited the characteristic hypermotility response, whereas HANDU returned to control values. These data suggest that analgesic doses of morphine enhance cholinergic neuronal activity in the mouse by a mechanism that is specific to an opiate receptor.

The possibility that morphine (10 mg/kg, 20 mg/kg) increases HANDU into striatal synaptosomes via a direct action on the cholinergic nerve terminal was examined by incubating synaptosomes in the presence of pharmacologically

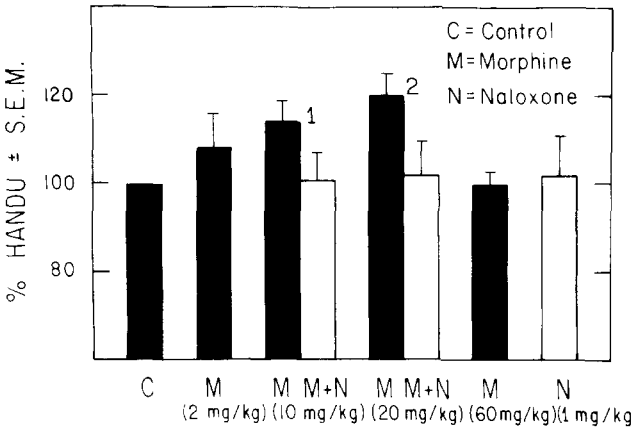

FIG. 2. Effects of morphine and naloxone on HANDU into striatal synaptosomes. $^1p=0.05$. $^2p=0.01$. All doses of morphine were administered IP 40 min prior to sacrifice. Naloxone was administered IP 60 min prior to sacrifice. The data represent Mean±S.E.M. of at least 4 separate determinations (n for control group = n for experimental group).

effective concentrations of morphine and measuring high-affinity choline transport. Table 1 demonstrates that 0.1 μM–10.0 μM morphine had no effect on HANDU into striatal synaptosomes.

Since it did not exert a direct effect on the cholinergic nerve terminal over a wide range of concentrations, the possibility that morphine (10 mg/kg, 20 mg/kg) indirectly activates the striatal cholinergic system through a mechanism involving the dopaminergic system was examined. If morphine is inhibiting nigrostriatal dopaminergic function, then one would observe an increase in cholinergic neuronal activity and choline uptake, and dopaminergic agonists should reverse this effect. Pharmacological support for this concept is presented in Table 2. The dopaminergic agonists, apomorphine (10 mg/kg) or amantadine (50 mg/kg) reversed the stimulatory effect of morphine on HANDU at doses that did not alter uptake when administered alone.

TABLE 1

EFFECT OF MORPHINE ON HANDU INTO SYNAPTOSOMES *IN VITRO*

Morphine Concentration	n*	% HANDU ± S.E.M
Saline	3	100.0
10.0 μM	3	105.6 ± 0.8
1.0 μM	3	101.1 ± 3.9
0.1 μM	3	97.8 ± 3.9

*N = Number of determinations.

TABLE 2

EFFECTS OF DOPAMINERGIC AGONISTS ON MORPHINE-STIMULATED HANDU INTO SYNAPTOSOMES

Treatment§	n*	Dosage (mg/kg)	% HANDU ± S.E.M.
Control	—	—	100.0
Morphine	8	10	114.5 ± 5.1†
Morphine	9	20	122.4 ± 5.6‡
Apomorphine	6	10	96.7 ± 6.7
Apomorphine + Morphine	5	10 + 10	101.7 ± 10.9
Apomorphine + Morphine	5	10 + 20	90.8 ± 5.1
Amantadine	4	50	105.6 ± 7.9
Amantadine + Morphine	4	50 + 20	95.9 ± 5.4

*n = Number of determinations (n for control group = n for experimental group).

†$p = 0.05$.

‡$p = 0.01$.

§Morphine was administered IP 40 min prior to sacrifice. Apomorphine and amantadine were administered IP 60 min prior to sacrifice.

DISCUSSION

A high-affinity Na^+-dependent choline transport system is localized in the membrane of cholinergic nerve terminals. This system is intimately associated with the conversion of transported choline to acetylcholine and may represent a rate-limiting step in ACh synthesis by cholinergic neurons. In addition, high-affinity Na^+-dependent choline transport into synaptosomes is coupled to neuronal activity. Administration of a wide variety of pharmacological agents that reduce ACh release and turnover rate also reduce HANDU into synaptosomes, whereas increases in HANDU are observed in response to the administration of compounds that increase ACh release and turnover rate. Thus, HANDU has been utilized as a qualitative measure of the activity of cholinergic neurons *in vivo* [1,10] for review, see [23].

Our experiments verify the use of HANDU as an index of cholinergic neuronal activity in the mouse corpus striatum. Parasympatholytics stimulate ACh release and synthesis [12,19] and atropine significantly stimulated HANDU into striatal synaptosomes. Haloperidol, a dopamine receptor antagonist that increases ACh release and synthesis [25,26] significantly increased choline transport into striatal synaptosomes.

There is a vast literature concerned with the effects of opiates on dopaminergic (for review, see [8]) and cholinergic (for review, see [4]) systems in the brain. However, variability between strain, drug dosages, modes of administration, time of day and other such factors limits the degree to which these data can be compared. In the present studies, advantage was taken of the fact that our laboratory has previously accumulated a considerable amount of pharmacological and behavioral data with morphine in the CF-1 strain of mice [24, 28–30]. This permits standardization of many variables and provides an improved basis for data interpretation.

Morphine-induced hypermotility and analgesia in CF-1 mice can be dissociated from each other depending upon the dose administered. In addition, these parameters are differentially affected by dopaminergic compounds. The hypermotility response to morphine emerges at a relatively large dose (60 mg/kg). Pretreatment with dopaminergic agonists facilitates the response, whereas pretreatment with dopaminergic antagonists diminishes it [29]. These data are consistent with reports of increased striatal dopamine release and cAMP levels [27] and decreased striatal ACh release [18] in mice that exhibit hypermotility. In the present study,

HANDU into striatal synaptosomes in response to 60 mg/kg of morphine was not significantly different from control values. A possible explanation for our failure to observe a reduction in HANDU into striatal synaptosomes in response to a dose of morphine that elicits hypermotility and that would be expected to decrease cholinergic neuronal activity is that the striatal high-affinity choline transport system has the capacity to increase choline uptake in response to an increase in cholinergic neurotransmission, but has a limited capacity to reduce HANDU in response to a decrease in cholinergic neurotransmission. Support for the latter alternative is provided by observations in our laboratory that several agents which are known to enhance ACh release and turnover rate in the striatum also enhance HANDU into striatal synaptosomes. However, we were unable to demonstrate a significant reduction in HANDU in response to the administration of dl-Dopa. Similarly, other investigators have reported increased HANDU into rat striatal synaptosomes after the administration of haloperidol, whereas apomorphine did not reduce striatal HANDU despite the fact that it decreases ACh turnover rate in the rat striatum [1]. In contrast, both increased and decreased choline transport into hippocampal and cerebral cortical synaptosomes have been observed in response to a variety of pharmacological agents [1], suggesting that the striatal choline transport system may be unique in its apparent uniphasic potential for regulating choline availability. Since striatal cholinergic interneurons are tonically inhibited by dopaminergic input from the nigrostriatal tract [7], ACh release and synthesis are usually suppressed. Possibly, regulation of choline availability plays an important role in ACh synthesis under circumstances where disinhibition of cholinergic neurons occurs and a minor role under conditions of reduced cholinergic neuronal activity.

In contrast to the relatively large dose of morphine that is required to elicit hypermotility, the ED 50 for morphine-induced analgesia in the CF-1 strain of mice is approximately 9 mg/kg [24]. Pretreatment with dopaminergic agonists inhibits morphine analgesia, whereas pretreatment with dopaminergic antagonists augments this response [28]. The possibility that analgesic doses of morphine indirectly enhance striatal cholinergic neuronal activity was supported in the present study. Administration of morphine (10 mg/kg, 20 mg/kg) significantly stimulated HANDU into striatal synaptosomes in a naloxone-reversible manner. The observed increase in choline transport was not due to a direct effect of morphine on the cholinergic nerve terminal as demonstrated in the *in vitro* experiments. Pretreatment with dopaminergic agonists reduced the stimulatory effect of morphine on HANDU into striatal synaptosomes. Thus, morphine activates the striatal cholinergic system at analgesic doses and both analgesia and HANDU are modulated by dopaminergic compounds. Possibly, low doses of morphine inhibit dopaminergic neurotransmission, resulting in disinhibition of striatal cholinergic interneurons and an increase in striatal cholinergic neurotransmission.

A thorough analysis of the acute effects of opiates in the corpus striatum must take into account the multiple localizations of opiate receptors in this region. Clearly, the preferential activation or inhibition of a specific neurotransmitter system will be dictated by the effective concentration of narcotic in the striatum and in nuclei that modulate striatal neurotransmitter activity.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to Elizabeth Kim for statistical analysis of the data.

REFERENCES

1. Atweh, S., J. R. Simon and M. J. Kuhar. Utilization of sodium-dependent high-affinity choline uptake *in vitro* as a measure of the activity of cholinergic neurons *in vivo*. *Life Sci.* **17:** 1535–1544, 1976.
2. Biggio, G., M. Casu, M. G. Corda, C. DiBello and G. L. Gessa. Stimulation of dopamine synthesis in caudate nucleus by intrastriatal enkephalins and antagonism by naloxone. *Science* **200:** 552–554, 1978.
3. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72:** 248–254, 1976.
4. Domino, E. F. Opiate interactions with cholinergic neurons. In: *Advances in Biochemical Psychopharmacology*, vol. 20, *Neurochemical Mechanisms of Opiates and Endorphins*, edited by H. H. Loh and D. H. Ross. New York: Raven Press, 1979, pp. 339–355.
5. Goldstein, A. and P. Sheehan. Tolerance to opioid narcotics. I. Tolerance to the "running fit" caused by levorphanol in the mouse. *J. Pharmac. exp. Ther.* **169:** 175–184, 1969.
6. Gray, E. G. and V. P. Whittaker. The isolation of nerve endings from brain: An electron microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat.* **96:** 79–87, 1962.
7. Guyenet, P. G., Y. Agid, F. Javory, J. C. Beaujouan, J. Rossier and J. Glowinski. Effects of dopaminergic receptor agonists and antagonists on the activity of the neo-striatal cholinergic system. *Brain Res.* **84:** 227–244, 1975.
8. Iwamoto, E. T. and E. L. Way. Opiate actions and catecholamines. In: *Advances in Biochemical Psychopharmacology*, vol. 20, *Neurochemical Mechanisms of Opiates and Endorphins*, edited by H. H. Loh and D. H. Ross. New York: Raven Press, 1979, pp. 357–407.
9. Khalsa, J. H. and W. M. Davis. Motility response to morphine and amphetamine during chronic inhibition of tyrosine hydroxylase or dopamine β-hydroxylase. *J. Pharmac. exp. Ther.* **202:** 182–191, 1977.
10. Kuhar, M. J. and L. C. Murrin. Sodium-dependent, high affinity choline uptake. *J. Neurochem.* **30:** 15–21, 1978.
11. Kuschinsky, K. Are cholinergic mechanisms involved in morphine effects on motility? *Naunyn-Schmiedeberg's Arch. Pharmac.* **281:** 167–173, 1974.
12. Lundholm, B. and B. Sparf. The effect of atropine on the turnover of acetylcholine in the mouse brain. *Eur. J. Pharmac.* **32:** 287–292, 1975.
13. Moroni, F., E. Peralta, D. L. Cheney and E. Costa. On the regulation of γ-aminobutyric acid neurons in caudatus, pallidus, and nigra: Effects of opioids and dopamine agonists. *J. Pharmac. exp. Ther.* **208:** 190–194, 1979.
14. Oliverio, A. and C. Castellano. Genotype-dependent sensitivity and tolerance to morphine and heroin, dissociation between opiate induced running and analgesia in the mouse. *Psychopharmacology* **39:** 13–22, 1974.
15. Patrick, G. A., W. L. Dewey, T. C. Spaulding and C. S. Harris. Relationship of brain morphine levels to analgesic activity in acutely treated mice and rats and in pellet-implanted mice. *J. Pharmac. exp. Ther.* **193:** 876–883, 1975.
16. Pert, C. B. and S. H. Snyder. Opiate receptor: Demonstration in nervous tissue. *Science* **179:** 1011–1014, 1973.
17. Pollard, H., C. Llorens-Cortes and J. C. Schwartz. Enkephalin receptors on dopamine neurons in rat striatum. *Nature* **268:** 745–747, 1977.

18. Racagni, G., A. Oliverio, F. Bruno, A. Maggi and F. Cattabeni. Dopamine and acetylcholine interactions in brain structures of mouse strains and different sensitivities to morphine. In: *Advances in Biochemical Psychopharmacology*, vol. 16, *Nonstriatal Dopaminergic Neurons*, edited by E. Costa and G. L. Gessa. New York: Raven Press, 1977, pp. 565–570.
19. Saelens, J. K., J. P. Simke, J. Schuman and M. P. Allen. Studies with agents which influence acetylcholine metabolism in mouse brain. *Archs int. Pharmacodyn. Thér.* **209:** 250–258, 1974.
20. Sharkawi, M. and A. Goldstein. Antagonism by physostigmine of the "running fit" caused by levorphanol, a morphine congener, in mice. *Br. J. Pharmac.* **37:** 123–128, 1969.
21. Simantov, R., M. J. Kuhar, G. W. Pasternak and S. H. Snyder. The regional distribution of a morphine-like enkephalin in monkey brain. *Brain Res.* **106:** 189–197, 1976.
22. Simon, E. J., J. M. Hiller and I. Edelman. Stereospecific binding of the potent narcotic analgesic [^{3}H]-etorphine to rat brain homogenate. *Proc. natn. Acad. Sci. U.S.A.* **70:** 1947–1949, 1973.
23. Simon, J. R., S. Atweh and M. J. Kuhar. Sodium-dependent high-affinity choline uptake: A regulatory step in the synthesis of acetylcholine. *J. Neurochem.* **26:** 909–922, 1976.
24. Spoerlein, M. T. and J. Scrafani. Effects of time and 8-azaguanine on the development of morphine tolerance. *Life Sci.* **6:** 1549–1564, 1967.
25. Stadler, H., K. G. Lloyd, M. Gadea-Ciria and G. Bartholini. Enhanced striatal acetylcholine release by chlorpromazine and its reversal by apomorphine. *Brain Res.* **55:** 476–480, 1973.
26. Trabucchi, M., D. L. Cheney, G. Racagni and E. Costa. Involvement of brain cholinergic mechanisms in the action of chlorpromazine. *Nature* **249:** 664–666, 1974.
27. Trabucchi, M., P. F. Spano, G. Racagni and A. Oliverio. Genotype-dependent sensitivity to morphine: Dopamine involvement in morphine-induced running in the mouse. *Brain Res.* **114:** 536–540, 1976.
28. VanderWende, C. and M. T. Spoerlein. Antagonism by dopa of morphine analgesia. A hypothesis for morphine tolerance. *Res. Communs. chem. Path. Pharmac.* **3:** 37–43, 1972.
29. VanderWende, C., M. T. Spoerlein and N. C. Luc. Studies on the role of dopaminergic systems in morphine-induced motor activity. Comparison with noradrenergic and cholinergic systems. *Res. Communs. chem. Path. Pharmac.* **11:** 79–88, 1975.
30. VanderWende, C. and M. T. Spoerlein. Morphine-induced catalepsy in mice. Modification by drugs acting on neurotransmitter systems. *Neuropharmacology* **18:** 633–637, 1979.
31. Yang, H.-Y., J. S. Hong and E. Costa. Regional distribution of leu and met enkephalin in rat brain. *Neuropharmacology* **16:** 303–307, 1977.