Pharmacology Biochemistry & Behavior, Vol. 32, pp. 563–567. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Effects of Naloxone and Naltrexone on Memory Consolidation in CD1 Mice: Involvement of GABAergic Mechanisms

CLAUDIO CASTELLANO,* INES B. INTROINI-COLLISON,† FLAMINIA PAVONE* AND JAMES L. McGAUGH†

**Istituto di Psicobiologia e Psicofarmacologia del C.N.R., via Reno 1, 00198-Roma, Italy*
†Center for the Neurobiology of Learning and Memory and Department of Psychobiology University of California, Irvine, Irvine, CA 92717

Received 20 June 1988

CASTELLANO, C., I. B. INTROINI-COLLISON, F. PAVONE AND J. L. McGAUGH. *Effects of naloxone and naltrexone on memory consolidation in CD1 mice: Involvement of GABAergic mechanisms.* PHARMACOL BIOCHEM BEHAV 32(2) 563–567, 1989.—The involvement of GABAergic mechanisms in the effects exerted by the opioid antagonists naloxone and naltrexone on memory consolidation was investigated in CD1 mice tested in a one-trial inhibitory avoidance task. In a first group of experiments posttraining administration of naloxone (2.0 and 4.0 but not 1.0 mg/kg) and naltrexone (0.5 and 1.0 but not 0.25 mg/kg), as well as those of the GABA-antagonists picrotoxin (0.5 and 1.0 but not 0.25 mg/kg) and bicuculline (0.25 and 0.5 but not 0.1 mg/kg) enhanced, whereas those of the GABA-agonist muscimol (1.0 and 2.0 but not 0.5 mg/kg) impaired retention on a 24-hr test. In a second group of experiments, picrotoxin, or bicuculline, administration enhanced, while muscimol treatment attenuated the effects of naloxone and naltrexone on retention. The results suggest that naloxone and naltrexone may influence memory consolidation in CD1 mice by interacting with the GABAergic system.

Bicuculline GABA Memory consolidation Inhibitory avoidance Mice Muscimol Naloxone Naltrexone Opioid antagonists Picrotoxin

RECENT investigations have demonstrated the involvement of GABAergic mechanisms in the effects of opioids (29). In particular, a variety of findings suggest that opioid antagonists may act as GABA-antagonists. For example, naloxone antagonizes the GABA-induced inhibition of neuronal firing in rats olfactory tubercle, and displaces (^{3}H)GABA from GABA binding sites in rat brain. In addition, naloxone enhances picrotoxin- or bicuculline-induced convulsions (10,31). Finally, the rate-decreasing effects of naloxone and picrotoxin on schedule-controlled responding in the pigeon are attenuated by drugs known to facilitate GABA-mediated synaptic inhibition, suggesting also, that this effect of naloxone is due to antagonism of GABA neurotransmission (3).

A number of experiments have shown that both GABAergic agents and opioid antagonists can influence learning and memory processes. In general memory improvement is seen following posttraining administration of both GABA- and opioid-antagonists and memory impairment is seen following the posttraining administration of GABA agonists. These effects have been observed in rats and mice tested in a variety of experimental conditions including one-trial inhibitory avoidance (2, 4, 8, 12–14, 16, 17, 20–22, 24, 28).

The present studies were designed to assess whether GABAergic mechanisms are involved in the effects exerted by the opioid antagonists on memory in the mouse. In these experiments naloxone and naltrexone were administered posttraining, either alone or in combination with the GABA antagonists picrotoxin and bicuculline or the GABA agonist muscimol, to CD1 mice trained in a one-trial inhibitory avoidance task.

METHOD

Subjects

Male CD1 mice (Charles River Labs., Como, Italy) weighing approximately 25 g were caged in groups of 8 with food and water available ad lib and maintained on a 12-hr light-dark cycle (lights on at 07:00) at a constant temperature of 21°C for two weeks prior to the experiments.

Apparatus and Procedures

The step-through inhibitory avoidance apparatus, similar to that previously described by Castellano and his colleagues (8), consisted of a 20×20×20 cm lucite box with black walls and a grid floor. A platform (12 cm long, 7.5 cm wide) extended from a small door (4×3 cm) in the front of the box. The box was placed at the edge of a table with the platform extending out from the table. The inside of the box was dark. A 40-W lamp was positioned 50 cm above the platform. Training and testing were performed between 14:00 and 17:00 hr. On the training trial the mouse was placed on the

platform facing away from the box. When the animal entered the box with all four feet the step-through latency was recorded, the entry was closed with a sliding door, and a footshock (0.7 mA, 1.0 sec, 50 Hz) was delivered. The mouse was then returned to its home cage. On the retention test 24 hr later the mouse was placed on the platform as on the training session and the step-through latency (maximum of 300 sec) was recorded.

The first series of experiments (A) examined the effects of posttraining administration of either naloxone or naltrexone. Different groups of mice were injected with naloxone (1.0, 2.0 and 4.0 mg/kg) or naltrexone (0.25, 0.5 and 1.0 mg/kg) immediately after training.

The second series of experiments (B) examined the effects of the posttraining administration of picrotoxin, bicuculline and muscimol. Different groups of mice were injected with picrotoxin (0.25, 0.5 and 1.0 mg/kg), bicuculline (0.1, 0.25 and 0.5 mg/kg) or muscimol (0.5, 1.0 and 2.0 mg/kg) immediately after training. An additional group of mice was injected with the bicuculline vehicle only. In both the first and second series of experiments, the highest dose of each drug was administered to an additional group of mice 120 min after training. The highest dose of the drugs was also administered immediately after training to other groups of mice which did not receive footshock.

A third series of experiments (C) examined the effects of posttraining injections of picrotoxin (0.25 mg/kg) or bicuculline (0.1 mg/kg) administered together with naloxone (1.0 mg/kg) or naltrexone (0.25 mg/kg). At these doses, these drugs had no effects when administered alone. In these experiments different groups of animals were injected with naloxone, or naltrexone, immediately after training, and 1 min later were injected with one of the GABA antagonists.

A fourth series of experiments (D) examined the effect of muscimol (2.0 mg/kg) administered together with naloxone (2.0 mg/kg) or naltrexone (0.5 mg/kg). In these experiments different groups of mice were injected with naloxone, or naltrexone, immediately after training, and were injected with muscimol 1 min later. For Experiments C and D, the retention performance of the animals was compared with that of a group given injections of saline both immediately and 1 min posttraining.

Naloxone (HCl), naltrexone (HCl) (ENDO, Garden City, NY), picrotoxin and muscimol (Sigma Chemical Corp., St. Louis, MO) were dissolved in saline (0.9%NaCl). Bicuculline (Sigma Chemical Corp., St. Louis, MO) was dissolved in a few drops of 0.1 N HCl, after which the final volume was made up with saline. The drug solutions were injected at a volume of 10 ml/kg. Saline was used for control treatments. All drugs were given intraperitoneally. Groups of 10 animals were used in all experiments.

The results were evaluated by ANOVA (1- and 2-way) in which the mean step-through latencies on the test day were compared (5). Further analyses for individual between treatment comparisons were carried out with post hoc tests (Duncan multiple range test).

RESULTS

Experiment A

As is shown in Table 1, immediate posttraining administration of naloxone or naltrexone significantly improved retention performance of mice. Separate ANOVAs (1-way) indicated that there were significant differences between the performances of both naloxone- and naltrexone-injected mice and that of mice injected with saline, $F(3,36)=23.39$ and 39.41 respectively, $p<0.001$. Individual between-treatment comparisons showed significant differences ($p<0.01$) between the performances of both naloxone- (2.0 and 4.0 but not 1.0 mg/kg) and naltrexone- (0.5 and 1.0 but not 0.25 mg/kg) injected mice and that of the saline-injected group.

TABLE 1

EFFECTS OF IMMEDIATE POSTTRAINING ADMINISTRATION OF NALOXONE AND NALTREXONE ON RETENTION OF A ONE-TRIAL INHIBITORY AVOIDANCE RESPONSE IN CD1 MICE

Treatment	mg/kg	Means (±SEM)
Saline		101.69 ± 4.23
Naloxone	1.0	103.19 ± 6.15
Naloxone	2.0	141.30 ± 5.18*
Naloxone	4.0	193.50 ± 15.30*
Naltrexone	0.25	105.19 ± 4.97
Naltrexone	0.5	139.39 ± 5.76*
Naltrexone	1.0	232.00 ± 17.26*

Mean step-through latencies (±SEM) recorded 24 hr after training. Groups of 10 animals.
*$p<0.01$ vs. saline.

Experiment B

As is shown in Fig. 1 retention performance was improved by posttraining injections of both picrotoxin and bicuculline, and impaired by muscimol. Separate ANOVAs (1-way) showed significant differences between the performances of picrotoxin-, bicuculline-, or muscimol-injected mice and that of the saline-injected group, $F(3,36)=44.35$, 55.22 and 136.83 respectively, $p<0.001$. Individual between treatment comparisons showed significant differences between the performances of picrotoxin- (0.5 and 1.0 but not 0.25 mg/kg), bicuculline- (0.25 and 0.5 but not 0.1 mg/kg) or muscimol- (1.0 and 2.0 but not 0.5 mg/kg) injected mice and that of the saline-injected group.

The performance of the animals injected with the bicuculline vehicle was not different from that of saline controls [retention scores (sec): saline: 101.3±3.3; bicuculline vehicle: 94.8±5.2].

The retention performance of the mice injected with naloxone (4.0 mg/kg), naltrexone (1.0 mg/kg), picrotoxin (1.0 mg/kg), bicuculline (0.5 mg/kg) or muscimol (1.0 mg/kg) 120 min after training did not differ from that of controls [retention scores (sec): saline: 99.2±6.3; naloxone: 98.3±8.2; naltrexone: 106.1±9.3; picrotoxin: 100.6±7.3; bicuculline: 102.5±5.2].

The same doses of the drugs were administered to animals that did not receive footshock on the training day. No difference was observed between their performance and that of saline-injected mice [retention scores (sec): saline: 6.6±1.2; naloxone: 8.4±2.2; naltrexone: 9.1±3.2; picrotoxin: 7.4±2.1; bicuculline: 6.3±3.2; muscimol: 8.2±1.4].

Experiments C and D

As is shown in Fig. 2 ineffective doses of naloxone and naltrexone, when injected together, significantly enhanced retention. These effects were not simply additive since the step-through latencies of mice injected with the higher doses

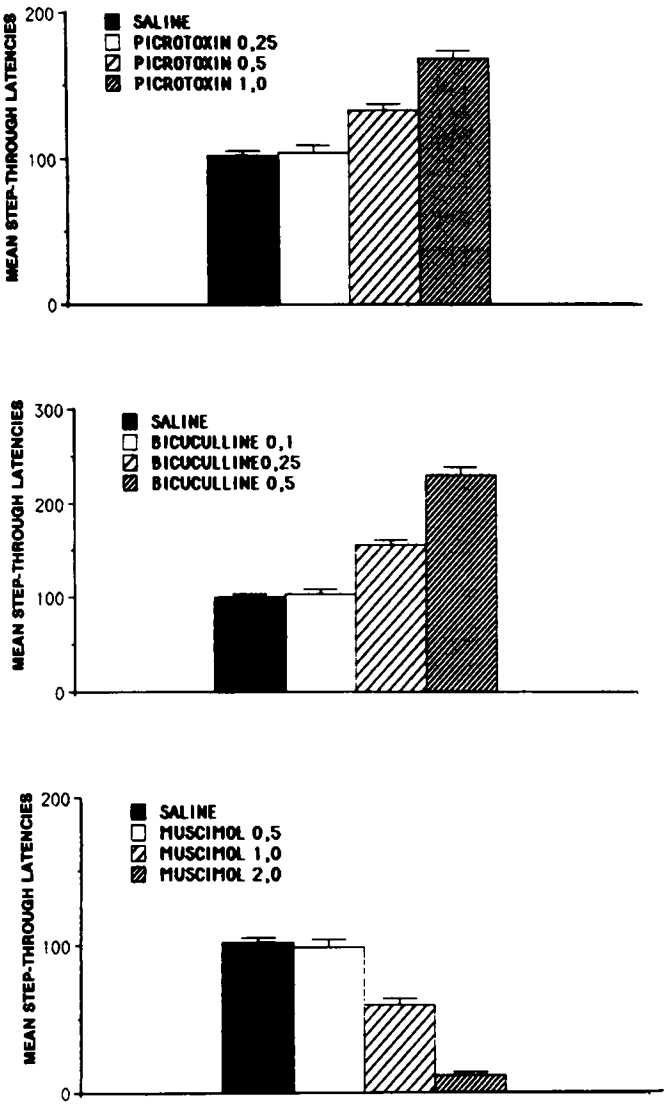

FIG. 1. Effects of immediate posttraining administration of picrotoxin, bicuculline and muscimol on retention of a one-trial inhibitory avoidance response in CD1 mice (mean step-through latencies ±SEM). Groups of 10 mice tested 24 hr after training.

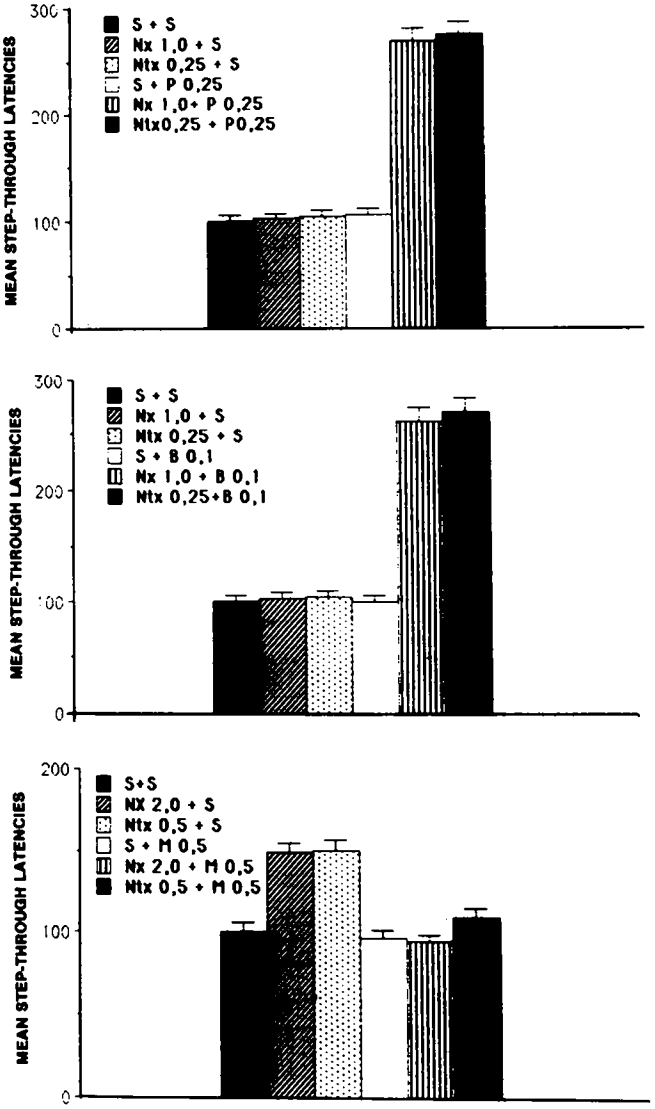

FIG. 2. Effects of immediate posttraining administration of naloxone (N—1.0 mg/kg), naltrexone (NTX—0.25 mg/kg) + saline (S), or in combination with picrotoxin (P—0.25 mg/kg), bicuculline (B—0.1 mg/kg) and muscimol (M—0.5 mg/kg) on retention of a one-trial inhibitory avoidance response in CD1 mice (mean step-through latencies ±SEM). S=saline-injected mice (2 injections). Groups of 10 mice tested 24 hr after training.

of each single drug were always significantly lower than those of the animals injected with two drugs. Further, muscimol attenuated the effects of both naloxone and naltrexone.

Separate ANOVAs (2-way) showed:

a) Significant main effects for both naloxone and naltrexone, and picrotoxin treatments [$F(1,36)=126.37$ and 136.05 respectively (naloxone), and 120.06 and 121.45 respectively (naltrexone), $p<0.01$], and significant naloxone × picrotoxin, and naltrexone × picrotoxin interactions, $F(91,36)=21.21$ and 108.39 respectively, $p<0.01$, were evident.

Individual between-treatment comparisons showed significant differences ($p<0.01$) between naloxone, or naltrexone + picrotoxin-injected mice and: a) naloxone- or naltrexone-injected mice, b) saline + picrotoxin-injected mice.

b) Significant main effects for both naloxone and naltrexone, and bicuculline treatments [$F(1,36)=96.99$ and 93.21 respectively (naloxone) and 123.63 and 111.97 respectively (naltrexone), $p<0.01$], and significant naloxone × bicuculline and naltrexone × bicuculline interactions, $F(1,36)=92.98$ and 111.70 respectively, $p<0.001$, were evident.

Individual between-treatment comparisons showed significant differences ($p<0.02$) between naloxone-, or naltrexone + bicuculline-injected mice and: a) naloxone- or naltrexone-injected mice, b) saline-injected mice.

c) Significant main effects for both naloxone and naltrexone, and muscimol treatments [$F(1,36)=18.06$ and 29.56 respectively (naloxone) and 28.75 and 15.30 respectively (naltrexone), $p<0.001$], and significant naloxone × muscimol and naltrexone × muscimol interactions, $F(1,36)=$

22.82 and 10.89 respectively, $p<0.01$, were evident.

Individual between-treatment comparisons showed significant differences ($p<0.01$) between naloxone-, or naltrexone + muscimol-injected mice and: a) naloxone- or naltrexone-injected mice, b) saline-injected mice.

DISCUSSION

The findings of the first two series of experiments (Experiments A and B) clearly show that the opioid antagonists naloxone and naltrexone, and the GABA antagonists picrotoxin and bicuculline improve memory consolidation in CD1 mice, whereas the GABA agonist muscimol exerts memory-impairing effects. All these actions were time-dependent: injections of the drugs 120 min after training were ineffective. Further, the effects were not due to nonspecific proactive pharmacological effects of the drugs lasting more that 24 hr. In the animals that did not receive footshock on the training day, the day-2 step-through latencies of the animals given posttraining drug injections did not differ from those of saline-injected controls. Further, the drugs did not affect step-through latencies when administered prior to training. The results confirm previous evidence obtained in rodents injected with these drugs and tested in one-trial inhibitory avoidance tasks, as well as in a variety of other experimental conditions (2, 4, 8, 12–14, 16, 17, 20–22, 24, 28).

The findings of the third and the fourth series of experiments (Experiments C and D) suggest the view that GABAergic mechanisms are involved in the effects of naloxone and naltrexone on memory consolidation in mice. Low doses of naloxone or naltrexone, and of the GABA antagonists picrotoxin or bicuculline, which had no effect on retention when administered alone, produced natural potentiation of retention when administered together following training. Further, muscimol treatment attenuated the effects of both opioid antagonists. These findings are consistent with extensive evidence from other behavioral, neurophysiological as well as neurochemical experiments suggesting that opioid antagonists act as GABA antagonists (3, 10, 18, 19, 27, 30, 31).

The GABA-antagonistic action of naloxone and naltrexone demonstrated by the present experiments may provide, at least in part, an understanding of the mechanisms underlying the antagonism by these opioid antagonists of the memory impairing effects of posttraining injections of the benzodiazepine, flunitrazepam (8), and ethanol (6). It is known that the benzodiazepines enhance GABA-mediated neurotransmission (9) and it has recently been demonstrated that ethanol displays GABA-agonistic effects (7). Finally, it should be noted that a number of experiments have demonstrated that cholinergic, dopaminergic and noradrenergic mechanisms are involved in the effects exerted by the opioid antagonists on memory consolidation in rodents (1, 11, 15, 20–22, 25). Inasmuch as GABA has been shown to interact with cholinergic as well as with catecholaminergic systems in the brain (26), it seems likely that GABA may also interact with these neurotransmitters in the modulation of memory.

ACKNOWLEDGEMENTS

This research was supported in part by USPHS Research Grant MH12526 and Contract N00014-87-K-0518 from the Office of Naval Research (to J.L.McG.).

REFERENCES

1. Baratti, C. M.; Introini, I. B.; Huygens, P. Possible interaction between central cholinergic muscarinic and opioid peptidergic systems during memory consolidation in mice. Behav. Neural Biol. 40:155–169; 1984.
2. Breen, R. A.; McGaugh, J. L. Facilitation of maze learning with posttrial injections of picrotoxin. J. Comp. Physiol. Psychol. 54:498–501; 1961.
3. Carter, R. B.; Leander, J. D. Comparison of the effects of naloxone and picrotoxin on schedule-controlled responding in the pigeon: possible GABA-antagonistic effects of naloxone. J. Pharmacol. Exp. Ther. 230:40–46; 1984.
4. Castellano, C. Strain-dependent effects of naloxone on discrimination learning in mice. Psychopharmacology (Berlin) 73:152–156; 1981.
5. Castellano, C. Dose-dependent modulation of memory by the enkephalin analog FK 33-824 in C57BL/6 mice. Behav. Neural Biol. 36:189–196; 1982.
6. Castellano, C.; Pavone, F. Naloxone-reversible effects of ethanol on passive avoidance behavior in mice. Physiol. Psychol. 11:291–295; 1983.
7. Castellano, C.; Pavone, F. Effects of ethanol on passive avoidance behavior in the mouse: Involvement of GABAergic mechanisms. Pharmacol. Biochem. Behav. 29:321–324; 1988.
8. Castellano, C.; Filibeck, U.; Pavone, F. Naltrexone-reversible effects of flunitrazepam on locomotor activity and passive avoidance behaviour in mice. Eur. J. Pharmacol. 104:111–116; 1984.
9. Costa, E.; Guidotti, A. Molecular mechanisms in the receptor action of benzodiazepines. Annu. Rev. Pharmacol. Toxicol. 19:531–545; 1979.
10. Dingledine, R.; Iversen, L. L.; Breuker, E. Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. Eur. J. Pharmacol. 47:19–27; 1978.
11. Fanelli, R. J.; Rosenberg, R. A.; Gallagher, M. Role of noradrenergic function in the opiate antagonist facilitation of spatial memory. Behav. Neurosci. 99:751–755; 1985.
12. Gallagher, M. Naloxone enhancement of memory processes: effects of other opiate antagonists. Behav. Neural Biol. 35:375–382; 1982.
13. Gallagher, M. Opiate antagonists improve spatial memory. Science 221:975–976; 1983.
14. Gallagher, M.; Kapp, B. S. Manipulation of opiate activity in the amygdala alters memory processes. Life Sci. 23:1973–1978; 1978.
15. Gallagher, M.; Rapp. P. R.; Fanelli, R. J. Opiate antagonist facilitation of time-dependent memory processes: dependence upon intact norepinephrine function. Brain Res. 347:284–290; 1985.
16. Grecksch, G.; Wetzel, W.; Matthies, H. Effect of n-dipropylacetate on the consolidation of a brightness discrimination. Pharmacol. Biochem. Behav. 9:269–271; 1978.
17. Grecksch, G.; Matthies, H. Differential effects of intrahippocampically or systemically applied picrotoxin on memory consolidation in rats. Pharmacol. Biochem. Behav. 14:613–616; 1981.
18. Gruol, D. L.; Barker, J. L.; Smith, T. G. Naloxone antagonism of GABA-evoked membrane polarizations in cultured mouse spinal cord neurons. Brain Res. 198:323–332; 1980.
19. Gumulka, S. W.; Dinnendahl, V.; Shonhofer, P. S. The effects of naloxone on cerebellar cGMP content. A possible GABA-antagonistic action? Naunyn Schmiedebergs Arch. Pharmacol. 306:169–172; 1979.
20. Introini, I. B. Participation de peptidos opioides endogenos en el proceso de la conlsolidacion de la memoria. Su posible interaccion con otros sistemas neuronales. Ph.D. Dissertation: Facultad de Farmacia y Bioquimica, University of Buenos Aires, 1984.

21. Introini-Collison, I. B.; Baratti, C. M. Opioid peptidergic systems modulate the activity of beta-adrenergic mechanisms during memory consolidation processes. Behav. Neural Biol. 46:227–241; 1986.
22. Introini-Collison, I. B.; McGaugh, J. L. Naloxone and beta-endorphin alter the effects of post-training epinephrine on memory. Psychopharmacology (Berlin) 92:229–235; 1987.
23. Introini, I. B.; McGaugh, J. L.; Baratti, C. M. Pharmacological evidence of a central effect of naltrexone, morphine and beta-endorphin and a peripheral effect of Met- and Leu-enkephalin on retention of an inhibitory response in mice. Behav. Neural Biol. 44:434–446; 1985.
24. Izquierdo, I. Effect of naloxone and morphine on various forms of memory in the rat: possible role of endogenous opiate mechanisms in memory consolidation. Psychopharmacology (Berlin) 66:199–203; 1979.
25. Izquierdo, I.; Graudenz, M. Memory facilitation by naloxone is due to release of dopaminergic and beta-adrenergic systems from tonic inhibition. Psychopharmacology (Berlin) 67:265–268; 1980.
26. Krogsgaard-Larsen, P.; Scheel-Kruger, J.; Kofod, H. GABA-neurotransmitters. Pharmacological, biochemical and pharmacological aspects. New York: Academic Press; 1979.
27. Mao, C. C.; Guidotti, A.; Costa, E. The regulation of cyclic guanosine monophosphate in rat cerebellum: possible involvement of putative amino acid transmitters. Brain Res. 79:510–514; 1974.
28. Messing, R. B.; Jensen, R. J.; Martinez, J. L., Jr.; Spiehler, V. R.; Vasquez, B. J.; Soumireu-Mourat, B.; Liang, K. C.; McGaugh, J. L. Naloxone enhancement of memory. Behav. Neural Biol. 27:266–275; 1975.
29. Oliverio, A.; Castellano, C.; Puglisi-Allegra, S. Psychobiology of opioids. Int. Rev. Neurobiol. 25:277–337; 1984.
30. Opmeer, F. A.; Gumulka, S. W.; Dinnendahl, V.; Shonhofer, P. S. Effects of stimulatory and depressant drugs on cyclic guanosine-3′,5′-monophosphate and adenosine 3′,5′-monophosphate levels in mouse brain. Naunyn Schmiedebergs. Arch. Pharmacol. 292:259–265; 1976.
31. Sagratella, S.; Massotti, M. Convulsant and anticonvulsant effects of opioids: relationships to GABA-mediated transmission. Neuropharmacology 21:991–1000; 1982.