Peptides, Vol. 12, pp. 1033–1037. © Pergamon Press plc, 1991. Printed in the U.S.A. 0196-9781/91 $3.00 + .00

Differential Effects of Selective Opioid Peptide Antagonists on the Acquisition of Pavlovian Fear Conditioning[1]

M. S. FANSELOW,*† J. J. KIM,* S. L. YOUNG,* D. J. CALCAGNETTI,† J. P. DECOLA,* F. J. HELMSTETTER† AND J. LANDEIRA-FERNANDEZ*

**Department of Psychology, University of California, Los Angeles, CA 90024*
†Department of Psychology, Dartmouth College, Hanover, NH 03755

Received 11 March 1991

FANSELOW, M. S., J. J. KIM, S. L. YOUNG, D. J. CALCAGNETTI, J. P. DECOLA, F. J. HELMSTETTER AND J. LANDEIRA-FERNANDEZ. *Differential effects of selective opioid peptide antagonists on the acquisition of Pavlovian fear conditioning.* PEPTIDES **12**(5) 1033–1037, 1991.—Pretreatment with opioid antagonists enhances acquisition of Pavlovian fear conditioning. The present experiments attempted to characterize the type of opioid receptor responsible for this effect using a procedure that assessed the fear of rats to a chamber previously associated with electric shock (1 mA, 0.75 s). Freezing, a species-typical immobility, was employed as an index of fear. Two μ opioid antagonists, CTOP (40 ng) and naloxonazine (10 μg), enhanced conditioning. On the other hand, the κ antagonist nor-binaltorphimine reduced conditioning. Two δ antagonist treatments (16-methyl cyprenorphine and naltrindole) had no reliable effect on acquisition. Thus the enhancement of conditioning appears to be mediated by μ receptors. Previous research has shown that the conditional fear produced by these procedures caused an analgesia that is also mediated by μ receptors. It is argued that the enhancement effect occurs because of an antagonism of this analgesia and that the analgesia normally acts to regulate the level of fear conditioning.

Opioid antagonist Pavlovian conditioning Fear Freezing Mu opioid receptor Delta opioid receptor Kappa opioid receptor Nor-binaltorphimine 16-Methyl cyprenorphine Naltrindole Naloxonazine Stress-induced analgesia $Cys^2Tyr^3Orn^5Pen^7$-amide

PAVLOVIAN fear conditioning occurs when a neutral stimulus is paired with an aversive event, which, in the laboratory, is typically a painful electric shock. The previously neutral stimulus rapidly acquires the ability to produce a fear reaction following such experience and is referred to as a conditional stimulus (CS). There are several behavioral manifestations of the conditional fear response to the CS [for an overview see (10)]. Two prominent aspects to the fear reaction are a cessation of ongoing activity, termed *freezing* (7), and a reduction in reactivity to painful stimuli, termed *conditional analgesia* (11). Examples of these reactions are provided by studies that placed rats in a distinctive chamber where they received a few brief electric shocks. Rats trained in this manner show pronounced freezing and a reduced reactivity to a painful formalin injection when returned to that chamber at a later time (9). Neither of these reactions are seen in a chamber that is not associated with shock. Thus, in this situation, both freezing and analgesia are conditional responses.

Endogenous opioids are implicated in performance of previously acquired conditional analgesia, as treatment with the opioid antagonists naloxone or naltrexone prior to formalin *testing* reverses conditional analgesia. The critical opioid receptors are in the central rather than the peripheral nervous system (3). Of the three major opioid receptor types, μ and δ, but not κ, receptors seem to be critical (2, 15, 17). On the other hand, endogenous opioids are not critical to performance of the freezing response because it is not impaired when opioid antagonists are given prior to testing (9,21).

However, the conditional analgesia produced by endogenous opioids appears to play an important attenuating role in the acquisition of Pavlovian fear conditioning. As conditional analgesia develops, it reduces the impact of the painful unconditional stimulus (US) and therefore reduces the US's ability to support conditioning (8). On the first trial of fear conditioning, there is no conditional analgesia, so the US is unopposed and supports a relatively large increment in conditional fear. However, as conditional analgesia develops over CS-US pairings, the impact of the US is progressively reduced. Therefore, each successive increment in conditioning is smaller. Eventually, the conditional analgesia is great enough to prevent any further increase in fear conditioning and acquisition becomes asymptotic. In this way, conditional analgesia serves as negative feedback to regulate the acquisition of conditional fear. Implications of this negative feedback system for conditioning theory have been discussed elsewhere (11,12). Negative feedback accounts for a very general pattern in the conditioning literature, that predicted USs are

[1]Portions of this article were presented at the 12th Annual Winter Neuropeptide Conference, Breckenridge, CO (February, 1991).

not as effective at reinforcing conditioning as unpredicted ones (30).

The major support for this negative feedback model comes from the finding that if naloxone or naltrexone is given prior to *training* with a painful US, conditional freezing is enhanced. This enhancement in freezing is quite similar to the enhancements observed with increased US intensity (8,13). Like conditional analgesia as measured with the formalin test, this enhancement of conditional freezing depends on central opioid synapses (14). However, it has not yet been determined which of the major opioid receptor types are involved in the enhancement effect. Therefore, the goal of the present series of studies was to determine whether μ, δ and/or κ receptors are responsible for the opioid antagonist's enhancement of Pavlovian fear conditioning as indexed by freezing. Experiment 2 assessed the contribution of the μ_1 subtype of the μ opioid receptor.

EXPERIMENT 1

The first experiment used opioid antagonist treatments selected to target only one of the three major opioid receptor types. The cyclic somatostatin analog, $Cys^2Tyr^3Orn^5Pen^7$-amide (CTOP), served as a μ antagonist. This octapeptide is purported to act as a selective μ opioid receptor antagonist with no somatostatin-like activity (18). At an intracerebroventricular (ICV) dose of 40 ng, Fanselow et al. (17) found that this drug reduced conditional analgesia as assessed by the formalin test as well as the analgesia produced by the highly selective μ opioid agonist [$D\text{-}Ala^2$,-$NMePhe^4$,$Gly\text{-}ol^5$]enkephalin (DAGO). However, this dose of CTOP had no effect on the analgesia produced by the highly selective δ agonist [$D\text{-}Pen^2$,$D\text{-}Pen^5$]enkephalin (DPDPE) nor that produced by the highly selective κ opioid agonist trans-3,4 dichloro-N-methyl-N-(2-(1-pyrrolidinyl) cyclohexyl benzeneacetamide (U50,488H). Thus 40 ng CTOP appears to be selective for μ receptors.

As a δ opioid antagonist, 5 μg of 16-methyl cyprenorphine (M80) was employed. This dose was able to reverse conditional analgesia on the formalin test (15). It also reversed the analgesia produced by the δ agonist (DPDPE) but had no effect on the analgesia produced by the μ (DAGO) or κ (U50,488H) agonists. For generality, a second δ antagonist, naltrindole (10 μg/rat), was used in the present study. Naltrindole is a potent and highly selective δ opioid antagonist (29) and completely blocks the analgesia produced by DPDPE but not DAGO (4).

Nor-binaltorphimine (N-BNI, 10 μg) served as a κ antagonist. This substance appears to be a highly selective antagonist for the κ receptor in vitro (28) and in vivo (32). Using the same formalin test procedures as with CTOP and M80, 10 μg of N-BNI completely reversed the analgesia produced by the κ agonist U50,488H but did not affect the performance of conditional analgesia (17).

As in previous work, fear conditioning was measured by the freezing response. Freezing is characterized by sustained periods of immobility that usually occur while the animal is in a crouching posture (7). Prior research has clearly indicated that in such situations freezing is a conditional response caused by the Pavlovian conditioning of fear to the apparatus cues present at the time of shock (7).

METHOD

Subjects

The subjects were 143 naive adult female hooded rats of Long-Evans descent (Blue Spruce Farms, Altamont, NY), born and raised in the UCLA Psychology Department colony. They weighed between 197 and 293 g at the time of surgery. While most of our previous research [e.g., (8, 13, 14, 16)] on this topic has used female rats, it should be noted that the basic effect is readily obtainable in males [e.g., (20)]. Animals were individually housed in hanging stainless steel cages with ad lib access to food and water and maintained in a colony room with 12:12-h light-dark cycle. Test procedures were conducted during the light phase of the cycle.

Surgery

Animals were anesthetized with 45 mg/kg of sodium pentobarbital and treated with atropine sulfate (0.12 mg/rat). With the skull leveled between lambda and bregma, a 22-ga stainless steel guide cannula (Plastic Products, Roanoke, VA) was stereotaxically implanted into the right lateral ventricle (coordinates used were 0.5 mm posterior to bregma, 1.5 mm lateral to midline, and 3.2 mm ventral to the surface of the cortex). All rats were given a minimum of seven days to recover following the surgery. During this time subjects were adapted to transportation, handled daily and each dummy cannula was removed and replaced with a clean one.

Drugs and Injection

N-BNI and naltrindole were purchased from Research Biochemicals Inc. (Natick, MA). CTOP was bought from Peninsula Laboratories (Belmont, CA). M80 was a gift from Dr. C. F. C. Smith (Reckitt and Colman). Each antagonist was dissolved in slightly acidic (pH = 5.5) sterile isotonic saline via ultrasound sonification. A 0.9% saline (pH adjusted to 5.5) served as the control injection. The ICV injections consisted of inserting a 28-ga internal cannula (Plastic Products, Roanoke, VA) so that it extended 0.5 mm beyond the guide cannula. Then a 6 μl injection volume was delivered at a rate of 2 μl/20 s. The injection cannula remained in place for at least 40 s after the infusion before being pulled out. A day before the experiment, subjects were injected ICV with saline (6 μl) to habituate them to being restrained during the injection procedure.

Apparatus

Four identical observation chambers (28 × 21 × 10.5 cm; Lafayette Instrument Co., North Lafayette, IN) were used in both the training and testing phases. Each chamber was placed inside of a sound-attenuating chest with a white light bulb (1820 bayonet bulb, 28 V) that allowed the experimenter to observe the subjects' behavior through a double panel 25 × 53 cm clear Plexiglas window in the front wall of the chest. A videocamera was mounted outside the observation chambers and the observer watched a monitor that was outside the experimental room. A ventilation fan secured to each chest supplied background noise (78 dB, A scale).

The floor of each chamber was composed of 18 stainless steel rods (4 mm diameter), spaced 1.5 cm center to center, and connected to a shock generator and scrambler (Lafayette Instrument Co., North Lafayette, IN). Ammonium hydroxide solution (5%) was used to clean the chambers before and after each rat.

Procedure

The experiment was conducted in a series of separate replications that were run at different times. On the training day, subjects were injected ICV with either CTOP (40 ng/rat), M80 (5 μg/rat), naltrindole (10 μg/rat), N-BNI (10 μg/rat) or vehi-

cle. The CTOP, M80 and naltrindole groups were placed in the observation chambers 10 minutes following the injection, whereas the N-BNI animals were placed in the chamber 30 minutes after the injection. These timing intervals were based on previously published studies (4, 15, 17) so that the drugs would have maximal effects at the time of conditioning. Injection of the vehicle into the control rats was timed to correspond to the subjects in the drug-injected groups. After 3 minutes in the chambers, three successive footshocks (1 mA, 0.75 s duration, 20 s apart) were presented. Animals were returned to their home cage 20 s after the last shock.

On the following day, each rat was placed for 8 min in the same chamber in which shock was administered the day before. Behavior was videotaped throughout the session. A time-sampling procedure was used to assess fear conditioned to the chamber. Four animals were observed concurrently, 1 in each of the four chambers. Every 2 s an observation of 1 of the 4 animals was made. Each animal was therefore scored once every 8 s. Behavior was judged to be freezing or not at the instant the sample was taken. Freezing was defined as the absence of any visible movement of the body and vibrissae except for movement necessitated by respiration. All other behaviors were scored as general activity. An experienced observer who was uninformed about the subject's drug treatment collected the data. Interobserver reliability coefficients for this technique are about .97.

Histology

At the conclusion of the experiment, the rats were overdosed with sodium pentobarbital and infused ICV with 2 μl of India ink. Approximately 5–15 minutes later, animals were perfused intracardially with saline (0.9%) followed by formalin (10%). The brains were removed and coronal sections were made along the cannula tract. Positive cannula placement was determined by the presence of ink throughout the ventricles by an experimenter who was not informed of the subjects' treatment. Seventeen rats were excluded from the analyses because positive cannula placement could not be verified.

RESULTS AND DISCUSSION

Vehicle-treated control rats froze 34% of the time. The control rats did not differ reliably as a function of either replication or infusion-test interval. The number of samples scored as freezing for each rat was converted to the percentage of the vehicle control mean for the replication that the rat belonged to. These percent control scores were subjected to an overall one-way analysis of variance, which indicated a reliable difference between groups, $F(4,121)=5.96$, $p<0.001$. The data for each antagonist, expressed as a percentage of control, is presented in Fig. 1. Each drug treatment was compared to the control mean by a Dunnett test with differences judged as reliable when they were at the 0.05 level of significance.

As can be seen in the figure, pretreatment with the μ antagonist CTOP nearly doubled the level of freezing. This statistically reliable enhancement of Pavlovian fear conditioning resembles that obtained with naloxone (8) and naltrexone (21). On the other hand, freezing was reliably attenuated by the κ antagonist pretreatment. This effect of N-BNI is consistent with the effects of peripheral injection of opioid antagonists with a high affinity for κ receptors (16).

M80 had no reliable effect on freezing. Given the ability of this dose of M80 to reverse fear-induced analgesia on the formalin test (15), that outcome was surprising. To ensure that this lack of effect was not artifactual, the experiment was replicated several times so that the M80 data point was represented by a large sample size (42 rats). In addition, a second δ antagonist, naltrindole, did not affect conditioning either. Thus it seems unlikely that δ opioid receptors are involved in the enhancement of conditioning reported previously. Rather, it seems that only μ opioid receptors are responsible for the enhancement of fear conditioning that is obtained with less selective opioid antagonists.

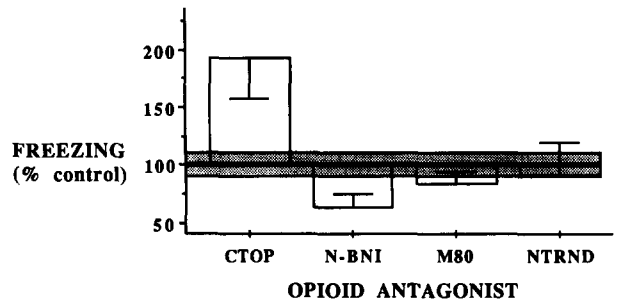

FIG. 1. The mean (±SEM) number of observations scored as freezing during testing is expressed as a percentage of the vehicle-injected controls (n=44) for $Cys^2Tyr^3Orn^5Pen^7$-amide (CTOP, n=8), nor-binaltorphimine (N-BNI, n=25), 16-methyl cyprenorphine (M80, n=42) and naltrindole (NTRND, n=7) treatment prior to training. The dark line represents the control mean and the shaded area is the SEM around that mean.

EXPERIMENT 2

The first experiment implicated μ receptors in the opioid antagonist-induced enhancement of Pavlovian conditioning. Experiment 2 sought to extend this observation to the chronic blockage of μ receptors produced by the long-acting opioid antagonist naloxonazine (NAZ-19). Centrally administered NAZ has been demonstrated to bind a subset of central μ receptors for a period greater than 24 h (27). This subset of μ opioid receptors, labeled μ_1, has been implicated in the central component of opiate analgesia (24). The present experiment tested the duration of the antagonism produced by NAZ by manipulating the interval between ICV administration of the antagonist and exposure to footshock. If NAZ is effective for 24 h, then administration of NAZ 24 h prior to shock should lead to an enhancement of conditioning. If NAZ is effective in producing such a long-lived enhancement of conditioning, it would implicate the μ_1 opioid receptor subtype in this phenomenon.

METHOD

Subjects and Surgery

The subjects were 46 rats, similar to those of Experiment 1, born and raised in the Dartmouth College Psychology Department colony. The subjects weighed 251–337 g at the time of testing. All subject variables, except the light-dark cycle (14/10 h light:dark cycle), were like those of the first experiment. The surgical procedures were similar to those of Experiment 1 except that the rats were anesthetized with ketamine hydrochloride (100 mg/ml/kg).

Procedure

The rats were assigned to one of 6 independent groups in a design that varied drug (NAZ or saline) and the interval between

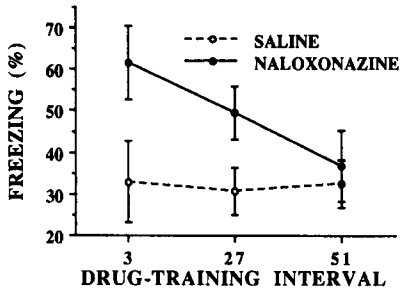

FIG. 2. The mean (±SEM) percentage of observations scored as freezing during an 8-min test given 24 h after training. The rats were pretreated with naloxonazine (10 μg/rat) or saline 3, 27 or 51 h prior to training.

ICV injection and shock (3, 27 or 51 h). The dose of NAZ was 10 μg/rat (injections consisted of 4 μl/40 s). The selection of this dose was based upon prior in vivo research (6).

Rats received shock either 3, 27 or 51 h after ICV injection. All conditioning and observations were conducted in one of four identical rodent chambers similar to those of Experiment 1 except that they measured 23.5 × 29 × 19.5 cm [described in detail by (9)]. Conditioning and testing were similar to that of Experiment 1. Each rat was placed into a chamber where, after 4 min, it received a total of three 1 mA/0.75 s footshocks, from a Grason-Stadler shock generator/scrambler, spaced 20 s apart. The rats were removed 20 s after the last shock and returned to the home cage. The next day the rats were placed into the same chamber where they were shocked the day before and their behavior was scored in the same manner as the previous experiment. Histology was identical to the previous experiment. All rats showed positive cannula placements.

RESULTS AND DISCUSSION

Figure 2 depicts the group means for the percentage of observations scored as freezing during the test session. A one-way analysis of variance indicated that the three saline groups were not reliably different from each other, $F(1,40)<1.0$. Therefore, the independent saline groups at each shock interval were combined and the mean of that combined control was compared to each drug-shock interval group by a Dunnett test with significance at the 0.05 level. The 3- and 27-h NAZ-treated groups were reliably different from control. However, the 51-h NAZ-treated group was not significantly different from control.

The central administration of NAZ (10 μg) either 3 or 27 h prior to conditioning resulted in an enhancement of freezing. The effect was similar to that obtained in Experiment 1 with CTOP given immediately prior to shock. When NAZ was given 51 h prior to shock there was no enhancement of freezing. This suggests that the opioid receptors responsible for the enhancement of fear conditioning were effectively blocked by NAZ for at least 27 h. The blockade at 27 h is consistent with an effect mediated at μ_1 receptors.

If the negative feedback model described in the Introduction were correct, the enhancement of conditioning seen after NAZ administration would indicate that this antagonist is acting on a population of receptors normally involved in the expression of conditional analgesia. Consistent with this suggestion, Pasternak and colleagues found that NAZ (10 mg/kg) effectively antagonized the analgesic actions of morphine sulfate (3.5 mg/kg) as indexed by the tail-flick assay (23). Similarly, follow-up observations in our laboratory revealed that analgesia produced by morphine sulfate (10 mg/kg), as measured by paw lick latency on the hot plate test, was significantly reduced in rats for at least 28 h after NAZ treatment (10 μg, ICV).

GENERAL DISCUSSION

Two μ opioid antagonists (CTOP and NAZ) enhanced acquisition of Pavlovian fear conditioning as indexed by freezing behavior. While δ antagonists (M80 and naltrindole) had no reliable effect on conditioning, a κ antagonist (N-BNI) interfered with acquisition. Since the drug was administered only during training, not testing, performance or state dependency accounts of the enhancement of freezing are untenable [see also (8)].

The enhancement of conditional freezing found with CTOP and NAZ was similar to that observed after the intraperitoneal administration of naloxone (13), naltrexone (21) and the ICV administration of quaternary naltrexone (14). Additionally, Hammer and Kapp (20) demonstrated that naloxone administered into ventrolateral but not the dorsal region of the periaqueductal gray (PAG) enhanced conditional freezing. Therefore, it seems likely that the antagonism of μ opioid receptors in the ventrolateral PAG is responsible for this enhancement effect.

According to the negative feedback model described in the Introduction, these μ opioid antagonists enhance conditioning by attenuating conditional analgesia. Consistent with this, the same dose of CTOP attenuated conditional fear-induced analgesia as measured with the formalin test of pain sensitivity (17). This dose of CTOP also reversed the analgesia produced by μ, but not δ or κ, opioid agonists. The ventrolateral PAG is an area associated with opioid analgesia (26) and recently, injections of naltrexone into ventrolateral PAG have been found to reverse conditional fear-induced analgesia as assessed with the formalin test (22). Therefore, there are anatomical and pharmacological parallels between the ability of opioid antagonists to enhance conditioning and their ability to reverse conditional analgesia. Since analgesia should reduce the aversiveness of shock and less aversive shocks do not condition well (13), it is quite parsimonious to attribute the enhancement of conditional fear-induced freezing and the reversal of conditional fear-induced analgesia to the same mechanism, that mechanism being antagonism of μ opioid receptors in the ventral PAG.

In an earlier paper (16), we reported that IP injections of the nonselective opioid antagonists, MR2266 and MR1452, reduced freezing and speculated that this was caused by their action at κ opioid receptors. The present finding, that the highly selective κ antagonist N-BNI also reduced freezing, strongly supports that conclusion. Since the present study used ICV administration, it seems likely that those κ receptors are central. It appears that disturbance of κ opioid systems by either agonists (31) or antagonists [present paper, (16)] will attenuate Pavlovian conditioning. Thus κ receptors appear to play a complex role in conditioning that is in need of further elucidation.

It was surprising that M80 failed to increase Pavlovian fear conditioning because the same dose of M80 used here attenuated conditional analgesia as assessed with the formalin test (15). The effect of M80 on the formalin test is clearly attributable to δ opioid receptors. An additional δ opioid antagonist, the highly selective naltrindole, even at the relatively high dose used (4), had no effect on conditioning. Thus, while δ opioid receptors play a role in the expression of conditional fear-induced analgesia as assessed by the formalin test, they do not appear to play a role in the acquisition of fear conditioning as do μ receptors. A second analgesic response, dependent on a δ receptor, also seems to be activated by fear. This δ-mediated conditional analgesia specifically attenuates responses to tissue-damaging tonic pain stimuli such as formalin. Further studies will be necessary

to locate this δ receptor but it is likely to be outside the PAG because of the paucity of δ receptors in this structure (25). That an additional δ receptor-mediated analgesia contributes to the fear-induced suppression of formalin-induced behavior is consistent with the suggestion that tonic pain is inhibited by mechanisms in addition to those responsible for more general pain inhibition (5).

Elsewhere (11), we have suggested that there are three distinct types of responses to a painful US: withdrawal reflexes (e.g., tail-flick), recuperative behaviors (e.g., licking the site of a formalin injection) and Pavlovian fear conditioning (e.g., freezing to shock-associated stimuli). Fear suppresses all three of these reactions [for a review see (12)], possibly by activating a general analgesic mechanism with a central μ opioid component. This notion provides a parsimonious account of μ opioid antagonists' effects on aversively motivated learning in a manner that integrates much of what is known about endogenous analgesic mechanisms (1) and Pavlovian conditioning (12).

ACKNOWLEDGEMENTS

We thank Drs. C. F. C. Smith (Reckitt and Colman) and E. F. Hahn (IAVEX Corp., Miami) for the gifts of 16-methyl cyprenorphine and naloxonazine, respectively. F.J.H. is now at University of Wisconsin-Milwaukee. J.L.-F. was supported by CNPq, Brazil. This research was supported by NSF grant #BNS-900820 to M.S.F.

REFERENCES

1. Basbaum, A. I.; Fields, H. L. Endogenous pain control systems: Brainstem, spinal pathways, and endorphin circuitry. Annu. Rev. Neurosci. 7:309–328; 1984.
2. Calcagnetti, D. J.; Fanselow, M. S.; Helmstetter, F. J.; Bowen, W. D. [D-Ala^2, Leu^5, Cys^6]Enkephalin: Short-term agonist effects and long-term antagonism at delta opioid receptors. Peptides 10:319–326; 1989.
3. Calcagnetti, D. J.; Helmstetter, F. J.; Fanselow, M. S. Quaternary naltrexone reveals the central mediation of conditional analgesia. Pharmacol. Biochem. Behav. 27:529–531; 1987.
4. Calcagnetti, D. J.; Holtzman, S. G. Delta opioid antagonist, naltrindole, selectively blocks analgesia induced by DPDPE but not DAGO or morphine. Pharmacol. Biochem. Behav. 38:185–190; 1991.
5. Dennis, S. G.; Melzack, P. Pain modulation by 5-hydroxytryptaminergic agents and morphine as measured by three pain tests. Exp. Neurol. 69:260–270; 1980.
6. Dray, A.; Nunan, L. Evidence that naloxonazine produces prolonged antagonism of central delta opioid receptor activity in vivo. Brain Res. 323:123–127; 1984.
7. Fanselow, M. S. Conditional and unconditional components of post-shock freezing. Pavlov. J. Biol Sci. 15:177–182; 1980.
8. Fanselow, M. S. Naloxone and Pavlovian fear conditioning. Learn. Motiv. 12:398–419; 1981.
9. Fanselow, M. S. Shock-induced analgesia on the formalin test: Effects of shock severity, naloxone, hypophysectomy and associative variables. Behav. Neurosci. 98:79–95; 1984.
10. Fanselow, M. S. What is conditioned fear? Trends Neurosci. 7:460–462; 1984.
11. Fanselow, M. S. Conditioned fear-induced opiate analgesia: A competing motivational state theory of stress-analgesia. Ann. NY Acad. Sci. 467:40–54; 1986.
12. Fanselow, M. S. Analgesia as a response to aversive Pavlovian conditional stimuli: Cognitive and emotional mediators. In: Denny, M. R., ed. Fear, avoidance, and phobias: A fundamental analysis. Hillsdale, NJ: Erlbaum; in press.
13. Fanselow, M. S.; Bolles, R. C. Naloxone and shock-induced freezing in the rat. J. Comp. Physiol. Psychol. 93:736–744; 1979.
14. Fanselow, M. S.; Calcagnetti, D. J.; Helmstetter, F. J. Peripheral versus intracerebroventricular administration of quaternary naltrexone and the enhancement of Pavlovian conditioning. Brain Res. 444:147–152; 1988.
15. Fanselow, M. S.; Calcagnetti, D. J.; Helmstetter, F. J. Delta opioid antagonist, 16-Me cyprenorphine, selectively attenuates conditional fear- and DPDPE-induced analgesia on the formalin test. Pharmacol. Biochem. Behav. 32:469–473; 1989.
16. Fanselow, M. S.; Calcagnetti, D. J.; Helmstetter, F. J. Modulation of appetitively and aversively motivated behavior by the kappa (κ) opioid antagonist MR2266. Behav. Neurosci. 103:663–672; 1989.
17. Fanselow, M. S.; Calcagnetti, D. J.; Helmstetter, F. J. The role of μ and κ opioid receptors in conditional-fear induced analgesia: The antagonistic actions of nor-binaltorphimine and the cyclic somatostatin octapeptide, Cys^{2}Tyr^{3}Orn^{5}Pen^7-amide. J. Pharmacol. Exp. Ther. 250:825–830; 1989.
18. Gulya, K.; Pelton, J. T.; Hruby, V. J.; Yamamura, H. I. Cyclic somatostatin octapeptide analogues with high affinity and selectivity toward mu opioid receptors. Life Sci. 38:501–505; 1986
19. Hahn, E. F.; Pasternak, G. W. Naloxonazine, a potent, long-acting inhibitor of opiate binding sites. Life Sci. 31:1385–1388; 1982.
20. Hammer, G. D.; Kapp, B. S. The effects of naloxone administered into the periaqueductal gray on shock-elicited freezing behavior in the rat. Behav. Neural Biol. 46:189–195; 1986.
21. Helmstetter, F. J.; Fanselow, M. S. Effects of naltrexone on learning and performance of conditional fear-induced freezing and opioid analgesia. Physiol. Behav. 39:501–505; 1987.
22. Helmstetter, F. J.; Landeira-Fernandez, J. Conditional hypoalgesia is attenuated by naltrexone applied to the periaqueductal gray. Brain Res. 537:88–92; 1990.
23. Ling, G. S. F.; Spiegel, K.; Lockhart, S. H.; Pasternak, G. W. Separation of opioid analgesia from respiratory depression: Evidence for different receptor mechanisms. J. Pharmacol. Exp. Ther. 232: 149–155; 1985.
24. Ling, G. S. F.; Spiegel, K.; Nishimura, S. L.; Pasternak, G. W. Dissociation of morphine's analgesia and respiratory depressant actions. Eur. J. Pharmacol. 86:487–488; 1983.
25. Mansour, A.; Khachaturian, H.; Lewis, M. E.; Akil, H.; Watson, S. J. Anatomy of CNS opioid receptors. Trends Neurosci. 11:308–315; 1988.
26. Morgan, M. M.; Liebeskind, J. C. Site specificity in the development of tolerance to stimulation-produced analgesia from the periaqueductal gray matter of the rat. Brain Res. 425:356–359; 1987.
27. Pasternak, G. W.; Wood, P. J. Minireview: Multiple mu opiate receptors. Life Sci. 38:1889–1898; 1986.
28. Portoghese, P. S.; Lipowski, A. W.; Takemori, A. E. Binaltorphimine and nor-binaltorphimine, potent and selective κ-opioid receptor antagonists. Life Sci. 40:1287–1292; 1987.
29. Portoghese, P. S.; Sultana, M.; Takemori, A. E. Naltrindole, a highly effective and potent non-peptide d opioid receptor antagonist. Eur. J. Pharmacol. 146:185–186; 1988.
30. Rescorla, R. A.; Holland, P. C. Behavioral studies of associative conditioning in animals. Annu. Rev. Psychol. 33:265–308; 1982.
31. Schindler, C. W.; Lamb, M. R.; Gormezano, I.; Harvey, J. H. Effects of morphine, ethylketocyclazocine, and N-allylnormetazocine on classical conditioning of the rabbit nictitating response. Behav. Neurosci. 100:647–651; 1986.
32. Takemori, A. E.; Ho, B. Y.; Naeseth, J. S.; Portoghese, P. S. Nor-binaltorphimine, a highly selective kappa-opioid antagonist in analgesic and receptor binding assays. J. Pharmacol. Exp. Ther. 246:255–258; 1988.