Pharmacology Biochemistry & Behavior, Vol. 17, pp. 1175–1179, 1982. Printed in the U.S.A.

Naloxazone and Pain-Inhibitory Systems: Evidence for a Collateral Inhibition Model

ANNETTE L. KIRCHGESSNER,* RICHARD J. BODNAR*[1]
AND GAVRIL W. PASTERNAK†

**Department of Psychology, Queens College, CUNY, Flushing, NY*
†George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Departments of Neurology and Pharmacology, Cornell University Medical Center, New York, NY

Received 15 March 1982

KIRCHGESSNER, A. L., R. J. BODNAR AND G. W. PASTERNAK. *Naloxazone and pain-inhibitory systems: Evidence for a collateral inhibition model.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1175–1179, 1982.—The analgesic responses following morphine and cold-water swims (CWS) can be dissociated from each other. Indeed, certain manipulations in rats such as hypophysectomy or D-phenylalanine injections decrease CWS analgesia while increasing morphine analgesia. The present study examined the reciprocal notion, namely whether a manipulation that decreases morphine analgesia would increase CWS analgesia. Naloxazone, an opiate antagonist which selectively inhibits the high affinity binding site in a long-acting manner, was administered intracerebroventricularly and assessed for its effects upon morphine analgesia and CWS analgesia as measured by the jump test. While intracerebroventricular injections of naloxazone reduced morphine analgesia at 0.5 and 24 hr following microinjection, the same 50 μg dose significantly increased CWS analgesia at 0.5 hr after injection, suggesting a mechanism of collateral inhibition between opioid and non-opioid pain-inhibitory systems.

Pain Analgesia Naloxazone Cold-water swims Morphine Collateral inhibition Rats

RECENT evidence has indicated that the analgesic responses to cold-water swims (CWS) and morphine can be dissociated from each other. These studies have provided support for the contention that separate opioid and non-opioid pain-inhibitory systems exist [3, 16, 25]. Indeed, marked examples of this dissociation occur in which some manipulations increase the potency of one analgesic response while decreasing the potency of the other. For instance, while the analgesic response to CWS is decreased in hypophysectomized animals or following D-phenylalanine administration [2,8], morphine analgesia is potentiated by these procedures [1, 4, 15]. Ehrenpreis and co-workers [13,14] have suggested that the pain-altering properties of D-phenylalanine are due to its action as an anti-enkephalinase. Therefore, a working model of the analgesic data would predict that while increased availability of endogenous opioids should decrease CWS analgesia while increasing morphine analgesia, decreased availability of endogenous opioids should potentiate CWS analgesia while decreasing morphine analgesia. Previous studies in our laboratory have not been able to verify the latter aspect of these predictions. Instead of the expected potentiations in CWS analgesia, morphine-tolerant animals display normal CWS analgesia [6] and animals pretreated with naloxone exhibit a dose-dependent, though non-significant, reduction in CWS analgesia [5]. However, naloxone and limited morphine tolerance may not be the perfect pharmacological tools with which to test the above hypothesis since they are only capable of competing successfully with endogenous opioids for some, but not all, populations of opiate receptors (see [18]).

Recently, Pasternak and colleagues [18, 20, 21, 26, 27, 28] have developed the opiate antagonist naloxazone which displays irreversible and selective effects upon high affinity (Kd <1 nM), but not low affinity (Kd 1–10 nM) opioid binding sites in both in vivo and in vitro situations. Behavioral observations reveal that naloxazone pretreatment decreases the analgesic potency of morphine, morphiceptin, ketocyclazocine, SKF 10,047, the enkephalins and beta-endorphin while producing little effect on morphine lethality [18, 19, 20, 21, 28]. Given naloxazone's highly specific effects upon wide classes of opiates and opiate receptors, it was thought that this compound could be employed to test whether decreased availability of endogenous opioids would potentiate CWS analgesia while decreasing morphine analgesia. Due to a limited supply of the compound, the present study initially compared the relative effectiveness of intracerebroventric-

[1]Requests for reprints should be addressed to Dr. R. Bodnar, Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

ularly-applied naloxazone or naloxone with vehicle to alter jump thresholds per se, and then further assessed central naloxazone effects upon morphine and CSW analgesia.

METHOD

Twenty-four male albino Sprague-Dawley rats weighing between 250 and 350 g were pretreated with chlorpromazine (3 mg/ml normal saline/kg body weight, IP) 20 min before anesthetization with Ketamine hydrochloride (75 mg/ml sterile water/kg body weight, IM). Each animal was stereotaxically implanted with a stainless steel 22 gauge guide cannula (Plastic Products) aimed 0.3 mm above the left lateral ventricle. With the incisor bar set at 5 mm, lateral ventricle coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal sutrue and 3.6 mm from the top of the skull. The guide cannula was affixed to three stainless steel anchor screws with dental acrylic. Ten days following surgery, nociceptive responsivity was determined by employing jump thresholds following electric shock which is described in detail elsewhere [5, 6, 7]. To determine whether naloxazone possessed any activity upon jump thresholds per se, groups of eight rats each received intracerebroventricular injections of either naloxazone at a dose of 50 μg (free base was dissolved with glacial acetic acid), naloxone at a dose of 50 μg (Endo Laboratories) or vehicle respectively. All pharmacological agents were dissolved in 5 μl of normal saline and infused by hand with a Hamilton microliter syringe at a rate of 1 μl every 15 sec through a stainless steel 28 gauge internal cannula which extended 0.5 mm ventral to the guide cannula. Jump thresholds were determined for each animal in each group immediately prior to the injection (baseline) and at 1 and 24 hr following the injection. The experimenter was uninformed as to the injection condition.

Thirty-two other rats, surgically prepared as described previously, were divided into four groups of eight rats each. Two of these groups received injections of naloxazone (50 μg) while the remainder received vehicle injections in the same manner as described above. Jump thresholds were determined immediately prior to the injection (baseline). One group of naloxazone-treated rats and one group of vehicle-treated rats then received subcutaneous injections of morphine sulfate at a dose of 5 mg/kg (5 mg morphine sulfate/ml buffered solution/kg body weight) at 0.5 and at 24 hr following the intracerebroventricular injections. Jump thresholds were determined 30 min after each morphine injection. Similarly, the other group of naloxazone-treated rats and the other group of vehicle-treated rats then were exposed to 3.5 min swims in a 2°C bath at 0.5 and 24 hr following the intracerebroventricular injections. Jump thresholds were determined 30 min after each swim. Again, the experimenter was uninformed as to whether naloxazone or vehicle was administered.

Following experimental testing, all animals were anesthetized with sodium pentobarbitol (100 mg/2 ml normal saline/kg body weight, IP) and perfused through transcardiac puncture with 0.9% saline followed by 10% buffered formalin. Each brain was removed, blocked, sliced into 40 μm sections, mounted and stained with cresyl violet for cell body visualization. Coronal sections through the lateral ventricle were analyzed under a light microscope for cannula tip localization. All animals were verified positively for cannula placements in the lateral ventricle.

TABLE 1

MEAN JUMP THRESHOLDS (SEM) OF RATS TREATED WITH NALOXAZONE, NALOXONE AND VEHICLE

Group		Baseline (BL)	Post-Injection (hr) 1	24
Naloxazone (50 μg)	mean	0.398	0.361	0.398
	SEM	0.020	0.020	0.013
	Difference from BL		−0.037	+0.000
Naloxone (50 μg)	mean	0.411	0.407	0.453
	SEM	0.017	0.016	0.016
	Difference from BL		−0.004	+0.042
Vehicle	mean	0.445	0.439	0.466
	SEM	0.022	0.018	0.024
	Difference from BL		−0.006	+0.021

RESULTS

Jump Thresholds

Table 1 indicates that significant differences were observed across groups, $F(2,21)=3.97$, $p<0.035$, across pre-injection and post-injection test times, $F(2,42)=6.91$, $p<0.003$, but not for the interaction between group and test time, $F(4,42)=0.98$. Vehicle-treated rats failed to exhibit differences between their pre-injection baseline jump thresholds and their jump thresholds elicited at 1 hr, $F(1,14)=0.13$ and 24 hr, ($F=1.48$) after injection. In contrast, naloxazone-treated rats displayed significant decreases in jump thresholds at 1 hr, $F=4.62$, $p<0.05$, but not at 24 hr ($F=0.00$) after injection relative to their pre-injection baseline values. Further, naloxone-treated rats displayed significant increases in jump thresholds at 24 hr ($F=6.01$, $p<0.05$), but not at 1 hr ($F=0.06$) after injection relative to their pre-injection baseline values.

The apparent effects of naloxazone and naloxone upon baseline pain thresholds were confounded however by the fact that the pre-injection baseline jump thresholds differed significantly across groups. This difference was due to an inadvertant and unfortunate misassignment of animals by the experimenter to the three groups in such a way that the pre-injection baseline thresholds of the vehicle group were significantly higher than the naloxazone group ($F=7.58$, $p<0.05$) and approached statistical significance when compared to the naloxone group ($F=3.84$). Therefore, in an attempt to equate these effects across groups and to determine whether naloxazone or naloxone induced jump threshold changes independent of baseline thresholds, difference scores were calculated by subtracting each post-injection value from its pre-injection counterpart. Analysis of these data revealed that while difference score values were significantly different between the two post-injection test times, $F(1,21)=17.94$, $p<0.001$, they failed to differ among groups, $F(2,21)=1.35$ or for the interaction between groups and test times, $F(2,21)=0.48$. Furthermore, post-hoc Scheffe comparisons indicated that jump threshold changes in

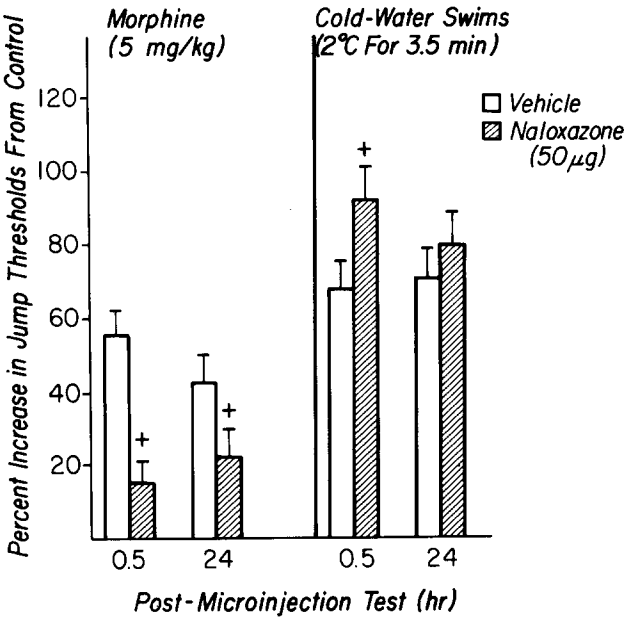

FIG. 1. The mean percent increase (SEM) in jump thresholds following morphine (5 mg/kg) administration (left panel) is depicted for animals that received either vehicle (baseline Mean=0.429) or a 50 μg dose of naloxazone (baseline Mean=0.427). Morphine analgesia was assessed 0.5 hr and 24 hr following the central injection. The mean percent increase (SEM) in jump thresholds following cold-water swims (2°C for 3.5 min) is depicted for vehicle-treated (baseline Mean=0.449) or naloxazone-treated (baseline Mean=0.455) rats. Cold-water swim analgesia was also assessed 0.5 hr and 24 hr following the central injection. Since all manipulations significantly increased jump thresholds above baseline levels (see text), the symbols denote a significant difference ($p<0.05$) in the magnitude of increase between the naloxazone and vehicle groups.

naloxazone-treated rats failed to differ from vehicle-treated rats at 1 hr (F=3.84) and 24 hr (F=0.78) after injection. Also, the jump threshold changes in naloxone-treated rats failed to differ from vehicle-treated rats at 1 hr (F=0.02) and 24 hr (F=1.76) after injection. Therefore, when the differences in baseline thresholds, which were caused by misassignments of individual animals into groups, were accounted for, neither naloxazone nor naloxone produced significant alterations in jump thresholds using these time and test parameters. A more controlled paradigm is necessary to confirm these observations.

Morphine Analgesia

The left panel of Fig. 1 illustrates naloxazone's suppressive effects upon morphine analgesia. Significant differences in jump thresholds were observed between groups, $F(1,14)=8.09$, $p<0.013$, across conditions, $F(2,28)=49.70$, $p<0.001$ and for the interaction between groups and conditions, $F(2,28)=12.66$, $p<0.001$. Pairwise comparisons revealed that morphine significantly increased jump thresholds above baseline values at 0.5 hr ($F=11.79$, $p<0.01$) and 24 hr ($F=15.53$, $p<0.01$) following naloxazone microinjection. Tolerance failed to develop between the two consecutive morphine injections. Morphine also significantly increased jump thresholds above baseline levels at 0.5 hr ($F=98.46$, $p<0.01$) and 24 hr ($F=59.10$, $p<0.01$) following vehicle microinjection. Significant tolerance effects ($F=5.00$, $p<0.05$) were observed for these two consecutive morphine injections. Though the pre-injection baseline values of vehicle-treated and naloxazone-treated rats failed to differ from each other (F=0.00), the analgesic responses following morphine of naloxazone-treated rats were significantly smaller than those observed for vehicle-treated rats at both 0.5 hr ($F=51.98$, $p<0.01$) and 24 hr ($F=14.76$, $p<0.01$) following the microinjection.

CWS Analgesia

The right panel of Fig. 1 illustrates naloxazone's potentiating effects upon CWS analgesia. Significant differences in jump thresholds were observed across conditions, $F(2,28)=53.74$, $p<0.001$, but not between groups, $F(1,14)=1.54$ or for the interaction between groups and conditions, $F(2,28)=1.10$. Pairwise comparisons revealed that CWS significantly increased jump thresholds above baseline values at 0.5 hr, $F(1,14)=57.34$, $p<0.01$ and 24 hr ($F=43.02$, $p<0.01$) following naloxazone microinjection. Adaptation failed to develop between the two swims (F=1.03). CWS also significantly increased jump thresholds above baseline values at 0.5 hr ($F=30.10$, $p<0.01$) and 24 hr ($F=33.00$, $p<0.01$) following vehicle microinjection, with adaptation again failing to develop (F=0.07). Though the pre-injection baseline thresholds of vehicle-treated and naloxazone-treated rats failed to differ from each other (F=0.01), the analgesic response following CWS of naloxazone-treated rats were significantly greater than those observed for vehicle-treated rats at 0.5 hr ($F=6.75$, $p<0.05$), but not at 24 hr (F=0.82) following the microinjection.

DISCUSSION

The present study showed that intracerebroventricular administration of a 50 μg dose of naloxazone reduced morphine analgesia by 72% and 49% at 0.5 hr and 24 hr following naloxazone pretreatment. While these significant reductions appear to be robust, it must be noted that systemic naloxazone administration 24 hr prior to injections of either opiates or opioid peptides eliminates their analgesic activity [18, 19, 20, 21, 27, 28]. The relative differences in effectiveness between the two routes may be explained in terms of procedural, pharmacological and physiological variables. First, the systemic studies employed quantal analgesiometric measures which determined the presence or absence of effects by a single criterion [18, 19, 20, 21, 27, 28]. The determination of pain thresholds in the present study employed more sensitive graded measures. Second, the systemic studies utilized a subcutaneous route of administration and dose ranges between 200 and 250 mg/kg. In contrast, the present study employed an intracerebroventricular route of administration and a dose of 50 μg. Hence, a 1200:1 dose ratio was observed between systemic and central routes for effective effects. In comparison, naloxone possesses a 20:1 ratio in dose between systemic (100 μg—see [17]) and central (5 μg—see [11]) routes in reversing morphine analgesia. Third, while intracerebroventricular naloxazone may be unable to reach all opiate receptors mediating opiate analgesia, the higher systemic naloxazone doses may gain access through the circulatory system.

Concurrent with its reductions of morphine analgesia, naloxazone-treated rats failed to display the limited tolerance induced by the second morphine injection. It

should be emphasized that full tolerance to morphine's analgesic properties did not occur following the second morphine injection in vehicle-treated animals. However, a significant reduction in morphine's analgesic efficacy was noted. Despite the fact that naloxazone-treated rats displayed a morphine analgesia following the first morphine injection that was lesser in magnitude than that following the first and second injections in vehicle-treated rats, the naloxazone-treated rats showed comparable analgesic effects following the first and second morphine injections. To speculate about this point before doing further and more elaborate tolerance studies may be premature. However, this lack of tolerance development could be due to the parametric equivalent of a floor effect. Alternatively, since naloxazone effects are transitory, the analgesia induced by the second morphine injection may be due to the partial re-occupation of opiate receptors by morphine that were vacated by naloxazone. Yet, since systemic naloxazone still antagonizes morphine analgesia in opiate-naive rats 24 hr after injection, this latter interpretation must be viewed with caution.

In contrast to the naloxazone-induced reductions of morphine analgesia, central naloxazone injections produced a significant 38% potentiation of CWS analgesia 1 hr following administration. These data provide further support for the contention that levels of endogenous opioids are capable of modulating particular analgesic procedures differentially: increased availability of endogenous opioids (e.g., D-phenylalanine) potentiates morphine analgesia [1] while decreasing CWS analgesia [8]. In contrast, decreased endogenous opioid activity (e.g., naloxazone) potentiates CWS analgesia and decreases morphine analgesia. Two major points need to be addressed: (a) in what model system could endogenous opioids operate to modulate differentially different analgesic processes; and (b) why did naloxazone, but not either naloxone or morphine tolerance, act to produce the observed effects.

Given the level of dissociation between various forms of stress-induced analgesia and morphine analgesia, it has been proposed that separate opioid and non-opioid pain-inhibitory systems exist (see reviews: [3, 16, 25]). Admittedly using tautological reasoning, the existence of at least two pain-inhibitory systems would suggest that they respond differentially or specifically to incoming environmental stimuli. Furthermore, given the maladaptive consequences if all systems designed to inhibit pain are activated in response to a particular nociceptive stimulus, it would be parsimonious if activation of one pain-inhibitory system could provide collateral inhibition upon the other pain-inhibitory system. Therefore, in this model of collateral inhibition, activation of one pain-inhibitory system (A) by endogenous or exogenous stimuli should inhibit the activity of the other (B), with the magnitude of effect dependent upon each system's tonic activational state and the magnitude of the activational stimuli. If we assume that the A system modulates morphine analgesia through the endogenous opioids, then A analgesia is enhanced by increasing endogenous opioid levels and reduced by decreasing them. Concurrently, B analgesia is modulated by endogenous opioid availability, albeit in an opposite manner. This part of the model expresses the present and previously reported [8] data. We also assume that the B system modulates non-opioid forms of analgesia, including that induced by CWS. However, the precise neurochemical mechanisms modulating these processes are not known. Yet, hypophysectomy reduces CWS analgesia [2] while potentiating morphine analgesia [4,15]. According to this model, the pituitary would then appear to be an important link in the B system not only to induce B analgesia, but also to inhibit the A system.

The strong form of this collateral inhibition hypothesis would predict that any manipulation that decreases the A form of analgesia should increase the B form and vice-versa. Important exceptions to this strong form exist since manipulations that decrease the analgesic response to CWS, such as genetic selection of Brattleboro rats deficient in vasopressin, fail to alter morphine analgesia [9]. Also, manipulations that decrease the analgesic response to morphine, such as parachlorophenylalanine injections or lesions placed in and around the periaqueductal gray [12, 22, 23, 24] fail to affect CWS analgesia [7,10]. Such exceptions may be explained by their respective involvement in the A and B systems to produce A analgesia and B analgesia, but their non-involvement in the collateral inhibitory component of the two systems. However, even if this latter explanation is true, why was naloxazone successful in potentiating CWS analgesia when either naloxone injections [5] or morphine tolerance [6] were not. The first possibility may be purely procedural, that is, the naloxone injections and morphine tolerance induction were made systemically while the naloxazone injection was central. The second possibility concerns the relative potencies and selective effects of naloxazone and naloxone respectively. Naloxazone selectively blocks the high affinity site irreversibly for a prolonged period of time whereas naloxone acts reversibly and with less specificity; i.e., it binds to a greater variety of binding sites [18, 20, 21]. Clearly, the notions that collateral inhibition is modulating different pain-inhibitory systems as well as the possible role of endogenous opioids in this modulation, will need further validation and refinement, but the model may serve to test the interplay between different analgesic processes.

ACKNOWLEDGEMENTS

This research was supported in part by NIH Grants NS14449 and GRSG 5SO5RR07064 (RJB) and DA02615 (GWP). CWP is a recipient of a Teacher-Investigator Award (NS00415). We thank Endo Laboratories for their generous gift of the naloxone hydrochloride.

REFERENCES

1. Alleva, E., C. Castellano and A. Oliverio. Effects of L- and D-amino acids on analgesia and locomotor activity of mice: their interaction with morphine. *Brain Res.* **198:** 249–252, 1980.
2. Bodnar, R. J., M. Glusman, M. Brutus, A. Spiaggia and D. D. Kelly. Analgesia induced by cold-water stress: attenuation following hypophysectomy. *Physiol. Behav.* **23:** 53–62, 1979.
3. Bodnar, R. J., D. D. Kelly, M. Brutus and M. Glusman. Stress-induced analgesia: neural and hormonal determinants. *Neurosci. Biobehav. Rev.* **4:** 87–100, 1980.
4. Bodnar, R. J., D. D. Kelly, A. Mansour and M. Glusman. Differential effects of hypophysectomy upon analgesia induced by two glucoprivic stressors and morphine. *Pharmac. Biochem. Behav.* **11:** 303–307, 1979.
5. Bodnar, R. J., D. D. Kelly, A. Spiaggia, C. Ehrenberg and M. Glusman. Dose-dependent reductions by naloxone of analgesia induced by cold-water stress. *Pharmac. Biochem. Behav.* **8:** 667–672, 1978.

6. Bodnar, R. J., D. D. Kelly, S. S. Steiner and M. Glusman. Stress-produced analgesia and morphine-produced analgesia: lack of cross-tolerance. *Pharmac. Biochem. Behav.* **8:** 661–666, 1978.
7. Bodnar, R. J., J. H. Kordower, M. M. Wallace and H. Tamir. Stress and morphine analgesia: alterations following parachlorophenylalanine. *Pharmac. Biochem. Behav.* **14:** 645–651, 1981.
8. Bodnar, R. J., M. Lattner and M. M. Wallace. Antagonism of stress-induced analgesia by D-phenylalanine, an anti-enkephalinase. *Pharmac. Biochem. Behav.* **13:** 829–833, 1980.
9. Bodnar, R. J., E. A. Zimmerman, G. Nilaver, A. Mansour, L. W. Thomas, D. D. Kelly and M. Glusman. Dissociation of cold-water swim and morphine analgesia in Brattleboro rats with diabetes insipidus. *Life Sci.* **26:** 1581–1590, 1980.
10. Brutus, M., D. D. Kelly, M. Glusman and R. J. Bodnar. Periaqueductal gray lesions and non-narcotic antinociception. *Soc. Neurosci. Abstr.* **5:** 606, 1979.
11. Dickenson, A. H., J. L. Oliveras and J. M. Besson. Role of the nucleus raphe magnus in opiate analgesia as studied by the microinjection technique in the rat. *Brain Res.* **170:** 95–111, 1979.
12. Dostrovsky, J. O. and J. F. W. Deakin. Periaqueductal gray lesions reduce morphine analgesia in the rat. *Neurosci. Lett.* **4:** 99–103, 1977.
13. Ehrenpreis, S., R. C. Balagot, J. E. Comaty and S. Myles. Naloxone reversible analgesia in mice produced by D-phenylalanine and hydrocinnamic acid inhibitors of carboxypeptidase A. In: *Advances in Pain Research and Therapy*, vol. 3, edited by J. J. Bonica, J. C. Liebeskind and D. G. Albe-Fessard. New York: Raven, 1979.
14. Ehrenpreis, S., R. C. Balagot, S. Myles, C. Advocate and J. E. Comaty. Further studies on the analgesic activity of D-phenylalanine (DPA) in mice and humans. In: *Endogenous and Exogenous Opiate Agonists and Antagonists*, edited by E. L. Way. New York: Pergamon, 1980.
15. Holaday, J. W., P. Y. Law, L. F. Tseng, H. H. Loh and C. H. Li. β-Endorphin: pituitary and adrenal glands modulate its action. *Proc. natn. Acad. Sci. U.S.A.* **74:** 4628–4632, 1978.
16. Lewis, J. W., J. T. Cannon and J. C. Liebeskind. Opioid and nonopioid mechanisms of stress analgesia. *Science* **208:** 623–625, 1980.
17. Martin, W. R. Opioid antagonists. *Pharmac. Rev.* **19:** 463–521, 1967.
18. Pasternak, G. W. Multiple opiate receptors: (^{3}H) ethylketocyclazocine receptor binding and ketocyclazocine analgesia. *Proc. natn. Acad. Sci. U.S.A.* **77:** 3691–3694, 1980.
19. Pasternak, G. W. Opiate, enkephalin and endorphin analgesia: relations to a single subpopulation of opiate receptors. *Neurology* **31:** 1311–1315, 1981.
20. Pasternak, G. W., S. R. Childers and S. H. Snyder. Opiate analgesia: evidence for mediation by a subpopulation of opiate receptors. *Science* **208:** 514–516, 1980.
21. Pasternak, G. W., S. R. Childers and S. H. Snyder. Naloxazone, a long-acting opiate antagonist: effects on analgesia in intact animals and on opiate receptor binding in vitro. *J. Pharmac. exp. Ther.* **214:** 455–482, 1980.
22. Samanin, R., D. Ghezzi, C. Mauron and L. Valzelli. Effect of midbrain raphe lesions on the antinociceptive action of morphine and other analgesics in rats. *Psychopharmacology* **33:** 365–368, 1973.
23. Samanin, R., W. Gulmulka and L. Valzelli. Reduced effect of morphine in midbrain raphe lesioned rats. *Eur. J. Pharmac.* **10:** 339–343, 1970.
24. Tenen, S. S. Antagonism of the analgesic effect of morphine and other drugs by p-chlorophenylalanine, a serotonin depleter. *Psychopharmacology* **12:** 278–285, 1968.
25. Watkins, L. R. and D. J. Mayer. The neural organization of endogenous opiate and non-opiate pain control systems. *Science* **216:** 1185–1192, 1982.
26. Wolozin, B. L. and G. W. Pasternak. Classification of multiple morphine and enkephalin binding sites in the central nervous system. *Proc. natn. Acad. Sci. U.S.A.* **78:** 6181–6185, 1981.
27. Zhang, A. Z., J. K. Chang and G. W. Pasternak. The actions of naloxazone on the binding and analgesic properties of morphiceptin (NH_2 Tyr-Pro-Phe-Pro-$CONH_2$), a selective mu-receptor ligand. *Life Sci.* **28:** 2829–2836, 1981.
28. Zhang, A. Z. and G. W. Pasternak. Opiates and enkephalins: a common binding site mediates their analgesic actions in rats. *Life Sci.* **29:** 843–851, 1981.