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Enhancement by Pain and Stress of Analgesia Produced by Epidural Sufentanil in the Rat

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This study examined whether pain or stress can enhance the analgesic effects of spinally administered opiates. The experiments determined the effects of mechanically produced pain and of the stress of being restrained on the analgesic effects of 0.63 μg of epidural sufentanil in rats using a tail-withdrawal procedure. The painful, as well as the stressful, conditions appeared to increase the duration of opiate analgesia 3.7- and 3.0-fold, respectively. The data offer initial evidence that pain and other stressful conditions can enhance the analgesia produced by spinally administered opiates. (Key words: Analgesics: sufentanil. Anesthetic techniques: epidural. Pain: experimental; mechanical; thermostimulation.)

RECENT STUDIES HAVE characterized the analgesic,[1] respiratory,‡ and other pharmacological effects[1,2] of epidurally administered opiates in the rat. The data obtained with the epidural rat preparation[3] are generally consistent with clinical evidence[4] that epidural opiates can produce satisfactory pain relief while exerting relatively few brain-mediated side effects. The rat data do not, however, correspond with patient data in terms of the duration of analgesia following epidural opiates; the absolute duration of analgesia in the rat appeared to be briefer than that in patients.[1] For example, a 40 μg per rat dose of morphine (*i.e.*, equivalent to approximately 10 mg in a 65-kg patient) had a duration of analgesic action of about 1 h. The duration of analgesic action of 10 mg of epidural morphine in patients is approximately 12–18 h.[5–7]

Several different explanations[1] can account for this difference in the duration of analgesic action of epidural opiates between rats and patients. One difference concerns the conditions to which the subjects are exposed at the time that analgesia is being assessed. Epidural opiates are typically given to patients who undergo surgical procedures and other manipulations that produce pain and stress. Pain[8,9] and other forms of stress[10,11] have been shown to enhance the analgesic effects of systemically administered opiates, and this enhancement consists, at least in part, of a prolongation of the duration of analgesia.[9,12]

The studies reported here examined whether the analgesia produced by the epidural opiate sufentanil[13] can be prolonged by mechanically produced pain or stress resulting from being restrained.

Materials and Methods

The experimental work described here conforms to the Guiding Principles in the Care and Use of Animals as approved by the Council of the American Physiologic Society and published in their Guide for Authors. Approval of this work was obtained from the Animal Care Committee.

The animals used were male Wistar rats weighing 250 ± 20 g. Rats were used only once. The laboratory room was air conditioned (21 ± 1° C; relative humidity 65 ± 5%).

EPIDURAL CATHETERIZATION

The technique of epidural catheter implantation is a slight modification of the technique described in detail elsewhere.[4] Briefly, under Thalamonal® (Innovar®; 2.5 mg droperidol and 0.05 mg fentanyl per ml; 1.5 ml per rat) and pentobarbital (3.5 $mg \cdot kg^{-1}$) anesthesia, a 0.5 cm length of polyethylene catheter (PE-10: O.D. 0.61 mm) was introduced in a cephaled direction into the epidural space *via* a hole drilled in the arch of the fourth lumbar vertebra. After fixation of the catheter to the vertebra with dental acrylic, the loose end was subcutaneously tunneled towards the neck region and blocked with a metal stopper. The animals were given about 1 week to recover from surgery. During this time, they were housed individually in standard rodent cages and had free access to food and tap water. Animals showing any sign of apparent neurological damage were discarded. After the experiments, all animals were killed by inhalation of CO_2, and the position of the tip of the catheter was examined on autopsy. Only the results of those animals whose catheter appeared to be located in the epidural space and in which there was no evidence of epidural fibrinous tissue reactions[14] surrounding the catheter and no perforation of the dura found, were used for data analysis. The method of epidural injection has been detailed elsewhere[3]; 20 μl of either sufentanil or saline was administered in increments of 1 μl, during an injection period of 60 s.

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‡ Van den Hoogen RHWM: In Vivo Pharmacological Effects of Epidural and Subcutaneous Opiates in the Rat. Academical Thesis, Leiden University, 1987, pp 71–85.

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Analgesic Assay

The tail withdrawal procedure used here has been described in detail elsewhere.[15] Briefly, the free hanging tail of the rat was immersed into a warm (55 ± 1° C) water bath, and the time for tail withdrawal was measured to the nearest 0.1 s. All readings were made by a single observer. The cut-off time was 10.0 s. Readings were taken once before and 5, 15, 30, 45, 60, and 90 min after an epidural injection was made.

Experimental Design

All rats that were not to be restrained were confined to an observation cage measuring 12 × 15 × 20 cm. Fourteen rats were restrained in a so-called Bollmann cage from 15 min before until 90 min after epidural injection. The cage consisted of a cylindrical rat holder (diameter: 4.5 cm) with a perspex front and back side —the latter with a hole for the tail—connected by eight stainless steel rods (ϕ 3 mm). Once mounted in this device, the animal can hardly move, although ventilation and movements of the free-hanging tail are not impaired.

In 14 other rats, acute pain was induced by placing an alligator clip (pressure ± 0.5 kg), which the animals were unable to remove, on each hindpaw from 5 min before until 90 min after epidural injection. The clips caused edema without skin perforation or inflammatory reactions.

Animals were randomly assigned to one of six groups (n = 7 per group). The three saline-treated groups were US (unrestrained, saline), RS (restrained, saline), and UPS (unrestrained, pain, saline). The three opiate-treated groups were UO (unrestrained, sufentanil), RO (restrained, sufentanil), and UPO (unrestrained, pain, sufentanil).

Drugs

Sufentanil citrate was freshly prepared as an aqueous solution. The dose (expressed as the base) used was 0.63 μg per rat. The selection of this dose was based on the results of earlier experiments.[1]

Statistical Methods

Data were analyzed by the Friedman two-way analysis of variance.[16] Differences between groups were evaluated using the Mann-Whitney U-test.[16]

Results

Overall Data Analysis

Friedman two-way analysis of variance[16] on the basis of the US, RS, UPS, UO, RO, and UPO groups revealed no difference in pre-injection latencies for tail withdrawal with condition of the rat. Post-injection latencies of the rats receiving saline did not differ with condition except at the 45-min interval ($P < .05$). The three sufentanil groups, however, differed significantly ($P < .05$, $P < .02$, $P < .05$, respectively) with condition at the 30-, 45-, and 60-min intervals, suggesting that the pain, stress, or both consistently modified analgesia following the 0.63-μg sufentanil dose.

Saline Control Data

In none of the 21 animals used in the three saline groups did the pre-injection latency for tail withdrawal exceed 6.0 s (fig. 1, left panel). Post-injection latencies in the unrestrained saline-treated group (US) also did not exceed this value. Therefore, and in accordance with earlier reports,[1,15] the 6.0-s level was chosen as the criterion to define analgesia in the present experiments.

Intrinsic Effects of Pain and Stress

In animals that received an epidural injection of saline and which were exposed to either stress (RS group) or pain (UPS group), post-injection latencies were slightly higher than in the unrestrained saline group. However, the median latency at no point exceeded 6.0 s, and the RS group differed significantly only ($P < .05$) from the US group at 15, 30, and 45 min post-injection (fig. 1).

Intrinsic Effects of Sufentanil

Epidural sufentanil, 0.63 μg, increased the median latency for tail withdrawal to a value greater than 6.0 s in all three groups (fig. 1, right panel). Comparisons with appropriate control data indicate (fig. 1) that the effect of sufentanil was significant ($P < .05$) in each case (UO, RO, and UPO compared with US, RS, and UPS, respectively).

Interaction of Pain and Stress with Opiate Analgesia

Stress and pain markedly enhanced the duration of analgesia of 0.63 μg of epidural sufentanil. That is, while the median post-injection latency in the UO group exceeded 6.0 s only for 5 and 15 min, the median latency continued to exceed 6.0 s for 60 min in both the RO and UPO groups. Differences with the UO data were significant ($P < .05$) at 30, 45, and 60 min after injection.

For each of the 42 rats, a plot was made of post-injection latency as a function of time after injection in the manner shown in figure 1. Duration of analgesia was defined as the period of time during which the polygon,

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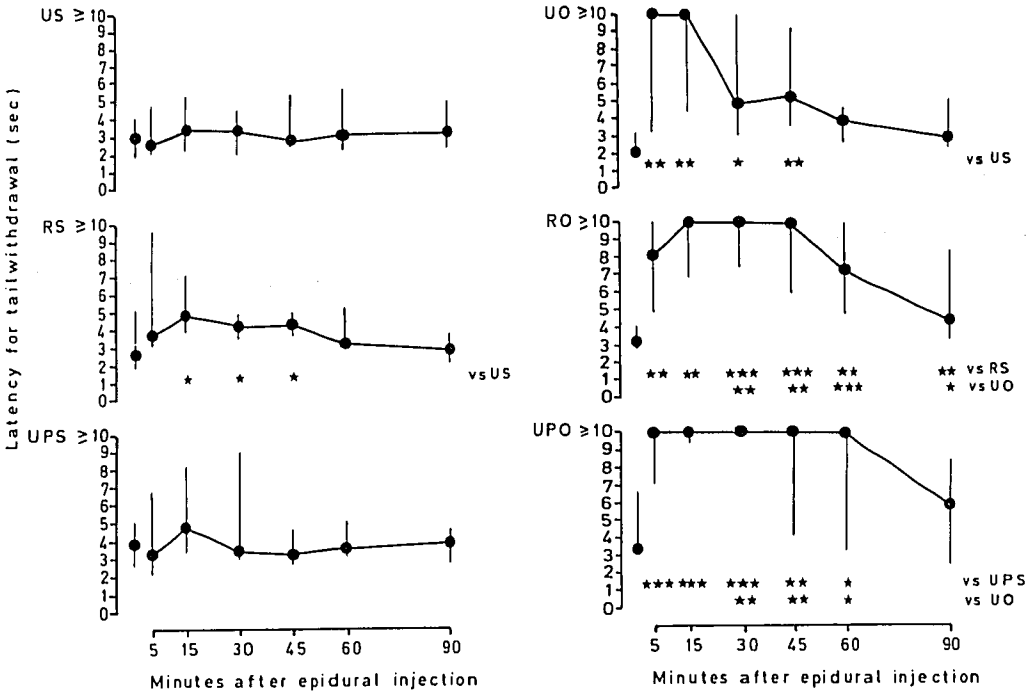

FIG. 1. Effects of pain and restraint stress on analgesia produced by epidural injection of 0.63 μg of sufentanil in rats. Animals were either unrestrained (U) or restrained (R), and received an epidural injection of saline (S) or the opiate drug sufentanil (O). Two groups of animals were unrestrained, but were exposed to pain (UP). Data points represent median latency and group range (n = 7) for tail withdrawal once before and at each time interval after injection. Asterisks indicate the two-tailed probability of differences between groups to be <.05 (*), <0.01 (**), or <.001 (***) (Mann-Whitney U-test).[16]

which connected post-injection latencies, exceeded 6.0 s.

The median duration of analgesia was 0 s in each of the three groups that received an epidural injection of saline (table 1). The median duration of the analgesia produced by sufentanil alone (UO group) was 24.3 min. This duration was increased to 73 min (*i.e.*, 3.0-fold) and to 89 min (*i.e.*, 3.7-fold) in the RO and UPO group, respectively ($P < .01$; table 1). The difference in duration of analgesia between the RO and UPO groups was not significant ($P > .05$).

Discussion

The experiments reported here offer initial evidence that the duration of analgesia produced by spinally administered opiates can be markedly enhanced by stress and pain. In the presence of stress and pain, the time during which analgesia was apparent increased from 24 to 73 and 89 min, respectively (table 1), after epidural injection of 0.63 μg of sufentanil.

That pain or stressful stimuli can produce analgesia[17–20] was confirmed by the increased latencies in the TWR test observed in saline-treated rats challenged with stress. That this analgesic effect was only significant in the stressed rats, and not in rats stimulated by pain, may be due to the small number of rats in each group. The observed 3.0- to 3.7-fold increase in the duration of opiate analgesia in the presence of respectively stress and pain agrees with earlier data[9] in which a similar mechanically produced pain increased the analgesic effects of systemic fentanyl 3.7-fold.

The mechanisms by which stress enhances opiate analgesia,[8–10] however, are poorly understood. There is

TABLE 1. Effects of Stress (R) and Pain (P) on the Duration of Analgesia Produced by Epidural Injection of Either Saline (S) or 0.63 μg of the Opiate Sufentanil (O) in Rats

Group	Duration of Analgesia in Min; Median Value (Limits)	Differences Between Groups				
		US	RS	UPS	UO	RO
US	0 (0–0)	—				
RS	0 (0–20.3)	N.S.	—			
UPS	0 (0–20.8)	N.S.	N.S.	—		
UO	24.3 (0–52.3)	†	*	*	—	
RO	73 (45–90)	‡	‡	‡	†	—
UPO	89 (40–90)	‡	‡	‡	†	N.S.

Data are reported as the median and limits of n = 7 per group. N.S. refers to differences not being significant.

Two-tailed probability of differences between groups: *$P < .05$, †$P < .01$, ‡$P < .001$ (Mann-Whitney U-test[16]).

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some evidence[11,12,21] that stress-induced enhancement of opiate analgesia may involve the release of endogenous opioids. The enhancement is independent of the pituitary-adrenocortical axis and is not associated with any detectable change in the pharmacokinetic fate of the opiate,[12] nor with an altered affinity of opiate binding sites for opiate ligands.[22] Stress enhances not only the analgesia, but also the changes in body temperature induced by opiates,[23,24] suggesting perhaps that the enhancement of analgesia represents a general potentiation of all opiate actions in the CNS. However, pain acts to attenuate, rather than enhance, the respiratory depressant effects of opiates[25] and of general anesthetics in patients, and recent data suggest that stress may markedly reduce the respiratory depressant effects of epidural sufentanil and morphine in rats.§ It thus seems that stress interferes in a selective manner with different opiate effects, *i.e.*, enhances some and attenuates others. The neuronal basis of this selectivity is not understood, but the finding that stress enhances the analgesic (fig. 1) while attenuating the respiratory depressant effects of epidural sufentanil in the rat argues in favor of the use of epidural opiates for the relief of pain.

It is generally thought[17,19] that the brain is the site of action for stress in producing analgesia and in enhancing analgesia produced by opiates. Specifically, stress of being restrained enhances analgesia produced by morphine administered into the cerebral ventricles.[26] Analgesia produced by spinal opiates[4] and, in particular, that produced by epidural injection of 0.63 μg of sufentanil in the rat[1,2] is likely to be mediated to a great extent by the spinal cord. The present findings that stress powerfully enhances opiate analgesia under these experimental conditions suggest, therefore, that the enhancement by stress of opiate analgesia does not require the opiate to act on the brain. This possibility is consistent with findings[27] that electrical stimulation of a peripheral nerve can produce a long-lasting and naloxone-reversible inhibition of the flexor reflex in spinal cats. It is also noteworthy, in this context, that a subpopulation of cell bodies in primary sensory ganglia bind radiolabelled opiates,[28-30] and that opiate receptors occur in the peripheral processes of primary sensory neurons (*i.e.*, in viscerosensory[29] and somatosensory nerves[30]).

In conclusion, the present findings indicate that stress of being restrained and mechanically produced pain both enhance the duration of analgesic action of epidural sufentanil approximately 3.7-fold. It appears that stress enhances some, and attenuates other, effects of opiates. This selective interference of stress with opiate effects involves actions that are mediated in the brain (*e.g.*, respiratory depression), but occurs also with the analgesia that epidural opiates produce by spinal mechanisms.

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References

1. Van den Hoogen RHWM, Colpaert FC: Epidural and subcutaneous morphine, meperidine (pethidine), fentanyl and sufentanil in the rat: Analgesia and other *in vivo* pharmacologic effects. ANESTHESIOLOGY 66:186–194, 1987
2. Colpaert FC, Leysen JE, Michiels M, Van den Hoogen RHWM: Epidural and intravenous sufentanil in the rat: Analgesia, opiate receptor binding, and drug concentrations in plasma and brain. ANESTHESIOLOGY 65:41–49, 1986
3. Van den Hoogen RHWM, Colpaert FC: Long term catheterization of the lumbar epidural space in rats. Pharmacol Biochem Behav 15:515–516, 1981
4. Cousins MJ, Mather LE: Intrathecal and epidural administration of opioids. ANESTHESIOLOGY 61:276–310, 1984
5. Magora F, Olshwang D, Eimerl D, Shou J, Katzenelson R, Coteu S, Davidson JT: Observations on extradural morphine analgesia in various pain conditions. Br J Anaesth 52:247–252, 1980
6. Torda TA, Pybus DA: A comparison of four opiates for epidural analgesia. Br J Anaesth 54:291–295, 1982
7. Nordberg G, Hedner T, Mellshand T, Dahlstrom B: Pharmacokinetic aspects of epidural morphine analgesia. ANESTHESIOLOGY 58:545–551, 1983
8. Colpaert FC: Can chronic pain be suppressed despite purported tolerance to narcotic analgesia? Life Sci 24:1201–1210, 1979
9. Colpaert FC, Niemegeers CJE, Janssen PAJ, Maroli AN: The effects of prior fentanyl administration and of pain on fentanyl analgesia: Tolerance to and enhancement of narcotic analgesia. J Pharmacol Exp Ther 213:418–424, 1980
10. Belenky GL, Holaday JW: Repeated electroconvulsive shock (ECS) and morphine tolerance: Demonstration of cross-sensitivity in the rat. Life Sci 29:553–563, 1981
11. Schlen H, Bentley GA: The possibility that a component of morphine-induced analgesia is contributed indirectly *via* the release of endogenous opioids. Pain 9:73–94, 1980
12. Appelbaum BD, Holtzman SG: Characterization of stress-induced potentiation of opioid effects in the rat. J Pharmacol Exp Ther 231:555–565, 1984
13. Niemegeers CJE, Schellekens KHL, Van Bever WFM, Janssen PAJ: Sufentanil, a very potent and extremely safe intravenous morphine-like compound in mice, rats and dogs. Arzneimittelforschung 26:1551–1556, 1976
14. Durant PAC, Yaksh TL: Epidural injections of bupivacaine, morphine, fentanyl and DADL in chronically implanted rats: A pharmacologic and pathologic study. ANESTHESIOLOGY 64:43–53, 1986
15. Janssen PAJ, Niemegeers CJE, Dony JGH: The inhibitory effects of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittelforschung 13:502–507, 1963
16. Siegel S: Non-Parametric Statistics for the Behavioral Sciences. New York, McGraw Hill, 1956, 312
17. Bodnar RJ, Kelly DD, Brutus M, Glusman M: Stress-induced analgesia: Neural and hormonal determinants. Neurosci Biobehav Rev 4:87–100, 1980
18. Hayes RL, Bennett GJ, Newton PG, Mayer DJ: Behavioral and

§ Unpublished data.

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physiological studies of non-narcotic analgesia in the rat elicited by certain environment stimuli. Brain Res 155:69–90, 1978
19. Kelly DD: Stress-induced analgesia. Ann NY Acad Sci 467:447, 1986
20. Madden J, Akil H, Patrick RL, Bonchas J: Stress-induced parallel changes in central opioid levels and pain responsiveness in the rat. Nature 265:358–360, 1977
21. Girardot MN, Holloway FA: Chronic stress affects the reactivity to morphine in young mature but not old rats. J Pharmacol Exp Ther 233:545–553, 1985
22. Appelbaum BD, Holtzman SG: Restraint stress enhances morphine-induced analgesia in the rat without changing apparent affinity of receptor. Life Sci 36:1069–1074, 1985
23. Martin GE, Pryzbylic AT, Spector NH: Restraint alters the effect of morphine and heroin on core temperature in the rat. Pharmacol Biochem Behav 7:463–469, 1977
24. Martin GE, Papp NL: Effect on core temperature of restraint after peripherally and centrally injected morphine in the Sprague-Dawley rat. Pharmacol Biochem Behav 10:313–315, 1979
25. Hanks GW, Twycross RG, Lloyd JW: Unexpected complications of successful nerve block. Morphine-induced respiratory depression precipitated by removal of severe pain. Anaesthesia 36:37–39, 1981
26. Appelbaum BD, Holtzman SG: Stress-induced changes in the analgesic and thermic effects of morphine administered centrally. Brain Res 358:303–308, 1985
27. Chung JM, Fang ZR, Cargill CL, Willis WD: Prolonged, naloxone-reversible inhibition of the flexor reflex in the cat. Pain 15:35–53, 1983
28. Ninkovic M, Hunt SP, Gleave JRW: Localization of opiate and histamine H_1-receptors in the primary sensory ganglia and spinal cord. Brain Res 241:197–206, 1982
29. Young III WS, Wamsley JK, Zoubin MA, Kuhar MJ: Opioid receptors undergo axonal flow. Science 210:76–78, 1980
30. Van der Kooy D: Hyperalgesic functions of peripheral opiate receptors. Ann NY Acad Sci 467:154–168, 1986

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