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RELEASE OF OPIOID PEPTIDES IN ANAESTHETIZED CATS?

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1 The effect on arterial blood pressure of intravenous injections of naloxone (200 μg) was examined in cats anaesthetized with chloralose. Usually these injections have no effect on blood pressure unless morphine or opioid peptides have been injected, when they produce a pressor response with tachycardia.

2 It was found that these injections produced a pressor response with tachycardia after a combination of two or more of the following surgical procedures: (1) tying sinus nerves, (2) removing stellate ganglia, (3) cutting vagi, (4) evisceration.

3 The pressor responses obtained in these conditions are taken as evidence that such procedures induce the release of endogenous opioid peptides.

4 The pressor responses to naloxone were greatest when all four surgical procedures had been performed and were then due to adrenaline secretion, evoked centrally by a sympathetic discharge to the adrenals.

5 If either the stellate ganglia or the viscera were left intact, but the remaining three surgical procedures performed, then the pressor responses to naloxone were due to a sympathetic discharge to adrenals and to blood vessels.

6 In cats that had received a subcutaneous injection of morphine (2 mg/kg) the adrenals played a minor role in the pressor responses to naloxone, unless the four surgical procedures had been performed. Then the adrenals became entirely responsible for them.

7 The opioid peptides released after the surgical procedures may be enkephalins or the C-fragment of lipotropin (β-endorphin). The stimulus for their release may be interruption of afferent sensory pathways from viscera or the 'stress' associated with the surgical procedures.

Introduction

The experiments to be described deal with a rise in arterial blood pressure obtained in anaesthetized cats on intravenous injection of naloxone. The first time the effect was obtained it was unexpected, because usually naloxone does not affect arterial blood pressure. If the cat had first been given an injection of either morphine or opioid peptides it would have been different because morphine as well as the opioid peptides would have lowered arterial blood pressure and slowed the heart, mainly by causing central inhibition of sympathetic tone to blood vessels and heart, and the morphine antagonist naloxone would then have reversed this effect producing a pressor response (Feldberg & Wei, 1977; 1978a, b). But neither morphine nor opioid peptides had been injected; instead the vagi had been cut and the stellate ganglia removed. The pressor response to naloxone may be explained on the assumption that the two operations had brought about a release of endogenous opioid peptides. The peptides have recently been isolated and described (Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975; Li & Chung, 1976; Bradbury, Smyth & Snell, 1976).

With the two operations, not only efferent sympathetic and parasympathetic fibres to the heart and other viscera were interrupted, but also the afferent sensory pathways from the viscera. It seemed more likely that it was the interruption of these afferent pathways that had brought about the peptide release. If so, it should be possible to increase the pressor response to naloxone by producing a greater release of peptides through more extensive interruption of afferent fibres from the viscera. To test this possibility the effect of intravenous injections of naloxone on blood pressure was compared in cats in which one or more of the following four surgical procedures had been performed: (1) cutting or tying-off the sinus nerves, (2) removal of stellate ganglia, (3) cutting the vagi, and (4) evisceration, i.e. removal of the entire gastro-intestinal tract with spleen and pancreas.

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Some of the results have been communicated to the Physiological Society (Dashwood & Feldberg, 1978; 1979).

Methods

The experiments were done on anaesthetized male cats weighing between 2.2 and 3.2 kg. Anaesthesia was induced by ethyl chloride followed by injection into a cephalic vein of 1% chloralose (65 mg/kg). The right femoral vein was cannulated for intravenous injections and the right femoral artery for recording arterial blood pressure on a Smith's Servoscribe potentiometric recorder with a transducer connected through a Cambridge pre-amplifier. For recording heart rate the blood pressure was recorded from time to time for about 10 s at high speed, so as to be able to count the individual oscillations in blood pressure due to the heart beat. The trachea was cannulated and the cats were artificially ventilated throughout the experiment with a Palmer respiratory pump.

In order to remove the stellate ganglia they were approached retro-pleurally from the space between the heads of the first and second rib, as described by Anderson (1904). To eviscerate the cats, i.e. to remove the whole of the abdominal part of the alimentary canal with spleen and pancreas, double ligatures were tied around the inferior mesenteric, the superior mesenteric and the coeliac artery near the aorta and around the portal vein, and the vessels cut between the ligatures. In most experiments care was taken to keep the hepatic artery patent; in some the hepatic artery was included in the ligatures of the coeliac artery. The results were the same. Strong double ligatures were tied around the stomach at its oesphageal, and around the large intestine at its rectal end. The gastrointestinal tract was then cut between the ligatures and removed. For cutting the splanchnic nerves and removal of the adrenals the approach in the eviscerated cats was from the opened abdominal cavity, otherwise retroperitoneally.

The naloxone used was the hydrochloride (Endo Laboratories, New York). All values given refer to the salt.

Results

In Figure 1, each filled circle gives the blood pressure effect of an intravenous injection of 200 μg of naloxone in a different cat. No pressor response occurred with naloxone in six non-operated cats, nor after tying-off the sinus nerves or removing the stellate ganglia, as shown in columns 1 to 3. A small pressor response occurred after cutting the vagi (shown in column 4): it was less than 20 mmHg and was not obtained in each cat. In contrast, if two of these surgical procedures, or all three, were performed together, naloxone regularly gave definite pressor responses, as seen from the columns 5 to 7. The responses obtained in cats that had been eviscerated and, in addition, had their vagi cut, or their vagi cut and their sinus nerves tied off are shown in columns 8 and 9. Evisceration appears to be more effective in releasing opioid peptides than does removal of stellate ganglia but not enough experiments have been carried out to be certain on this point. However, as shown in column 10, the greatest pressor responses, rises between 38 and 82 mmHg (mean 60 mmHg) were obtained with all four operations performed together; this suggests that the greatest release of opioid peptides had occurred in this condition.

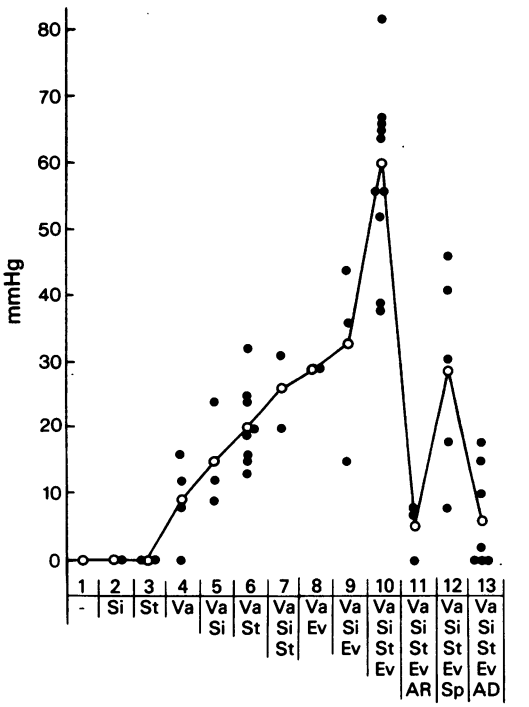

Figure 1 Effects of surgical procedures on blood pressure responses produced by a first intravenous injection of 200 μg of naloxone in cats anaesthetized with chloralose and artificially ventilated. The ordinates refer to the rises in arterial blood pressure (pressor responses) in mmHg produced by the naloxone injections. Each (●) represents the response in a different cat, and the position of the circle along the abscissae, within one of the 13 columns, indicates the surgical procedures undergone by the cat; (○), mean values. The abbreviations refer to the surgical procedures: (—) none; (Si) sinus nerves tied or cut; (St) stellate ganglia removed; (Va) vagi cut in the neck; (Ev) evisceration; (AR) adrenals removed; (Sp) splanchnic nerves cut; (AD) adrenals completely denervated.

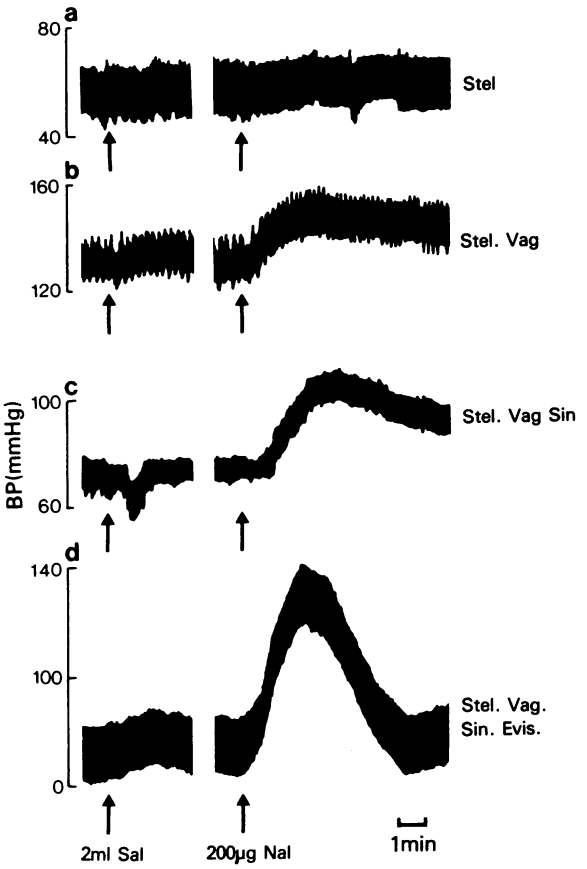

Figure 2 Arterial blood pressure from four cats anaesthetized with chloralose and artificially ventilated. The first arrows indicate intravenous injections of 2 ml 0.9% w/v NaCl solution, the second arrows intravenous injections of 200 µg of naloxone: (a) is a record from a 2.5 kg cat with stellate ganglia removed; (b) from a 2.9 kg cat with stellate ganglia removed and vagi cut; (c) from a 2.7 kg cat with stellate ganglia removed, vagi cut and sinus nerves tied; (d) from a 2.7 kg cat with stellate ganglia removed, vagi cut, sinus nerves tied and evisceration performed. Ordinates: arterial blood pressure in mmHg.

Blood pressure records of some of these results are shown in Figure 2. Record (a) is from a cat with stellate ganglia removed; naloxone produced no pressor response. A small but definite pressor response was obtained in record (b) from a cat with stellate ganglia removed and vagi cut. A greater response to naloxone occurred in record (c) from a cat in which, in addition, the sinus nerves had been tied off, and an even greater response, a rise of over 60 mmHg with acceleration of heart rate, in record (d) from a cat which had been eviscerated as well.

In one cat in which, instead of evisceration, the splanchnic nerves were divided in addition to removing the stellate ganglia, tying off the sinus nerves and cutting the vagi, the naloxone injection produced a rise in mean pressure of 36 mmHg.

The pressor response to naloxone did not decline with time after the surgical procedures. Of the five pressor responses over 60 mmHg shown in column 10 of Figure 1, three were obtained when the interval between the end of the surgical procedures and the injection of naloxone was longer than 30 min, the greatest response, a rise of 82 mmHg, being obtained after an interval of 5 h. This response is reproduced in Figure 3a, whereas Figure 3b illustrates, for comparison, a pressor response of 64 mmHg to naloxone injected 20 min after the end of the surgical procedures. In both experiments the pressor response was associated with some tachycardia. Heart rate, which was counted a few minutes before the naloxone injection and at the height of the pressor response increased from 298 to 334 beats/min in (a), and from 240 to 262 in (b).

With repeated intravenous injections of 200 µg of naloxone the pressor responses became attenuated whether the intervals between the injections were short or long. In the experiment illustrated in Figure 2d in which the first injection had produced a rise of 64 mmHg, a second one given 1 h later produced a rise of 44 mmHg; and in the first experiment (a) of Figure 3, in which the first injection of naloxone given 300 min after the end of the surgical procedures had produced a rise of 82 mmHg, three injections subsequently given at intervals of 15, 15 and 140 min produced rises of 34, 24 and less than 20 mmHg respectively.

Role of adrenals in the pressor response to intravenous naloxone

The finding that the greatest pressor response to naloxone was obtained in those cats in which all four surgical procedures had been performed indicating the greatest release of opioid peptides, posed the question of where the released peptides might act in this condition. Usually they would inhibit sympathetic tone to blood vessels and the heart, but after removal of the stellate ganglia and evisceration, the sympathetic innervation of the heart has gone and few innervated blood vessels are left. However, there is one tonic sympathetic innervation not interfered with by these operations, that to the adrenals which also appears to be inhibited by the opioid peptides, and therefore reversed by naloxone.

The strong pressor responses obtained in eviscerated cats with stellate ganglia removed, vagi cut and sinus nerves tied, were due to adrenaline release and therefore no longer obtained after removal of the

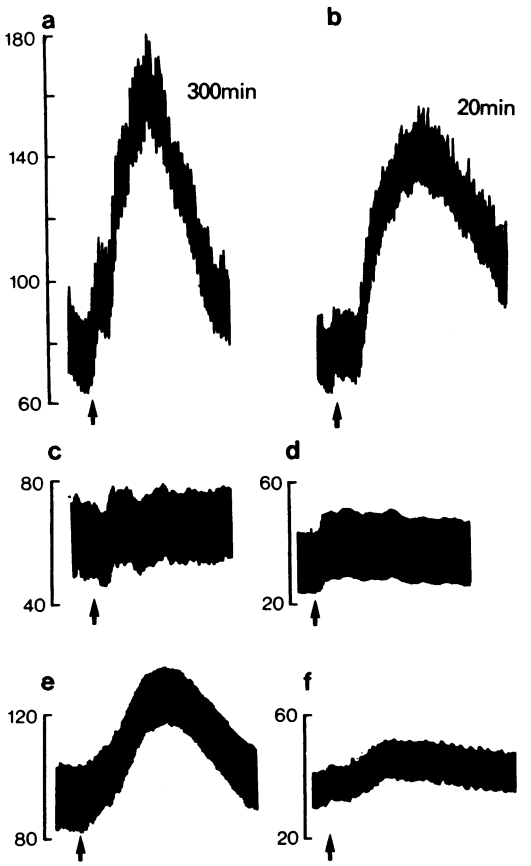

Figure 3 Arterial blood pressure in mmHg obtained from six cats weighing between 2.7 and 3.2 kg, anaesthetized with chloralose and artificially ventilated. All cats were eviscerated, had their stellate ganglia removed, vagi cut and sinus nerves tied. In addition, the two cats from which the records (c) and (d) were obtained had their adrenals either removed (c) or completely denervated (d), and the two cats from which (e) and (f) were obtained had their splanchnic nerves cut. The arrows indicate intravenous injections of 200 μg naloxone and the minutes given at the side of the pressor responses in (a) and (b) refer to the interval between end of surgical procedures and the naloxone injection.

adrenals, as is shown in column 11 of Figure 1. The effect on the adrenals could be a central or a peripheral one. When the splanchnic nerves were cut the pressor response was not abolished but was reduced to a different degree in different cats, as seen from the results in column 12 of Figure 1. This might suggest that the effect was partly central, partly peripheral. But the adrenals are not completely denervated when the splanchnic nerves are cut. As observed by T.R. Elliott as far back as 1913, they receive an additional sympathetic innervation via the upper lumbar paravertebral ganglia of the sympathetic chain. After removal of this innveration as well (the authors are grateful to Dr Marthe Vogt for having reminded them of this additional sympathetic innervation to the adrenals) practically no pressor response was obtained with naloxone. This is shown in column 13 of Figure 1. The fact that in some of these experiments small pressor responses of less than 20 mmHg were obtained suggests that a few sympathetic fibres to the adrenals may have escaped denervation. In addition, the sympathetic innervation to the skin and muscle vessels of the lower part of the body would not have been interrupted by any of the operations. It might account for the rise of less than 10 mmHg in two of the experiments given in column 11.

Blood pressure records of some of the results summarized in columns 11 to 13 are given in Figure 3c, d, e and f. The cats had undergone the same surgical procedures as the cats from which records (a) and (b) were obtained, but naloxone no longer produced a pressor response in (c) and (d) because, in addition, the adrenals had been removed in one, and completely denervated in the other cat. Records (e) and (f) were obtained from two other cats in which the splanchnic nerves were cut instead of fully denervating the adrenals. The difference in the size of the two pressor responses reflects the variability in the contribution made by the splanchnic nerves in the innervation of the adrenals.

In cats in which, of the four operations, either evisceration or removal of the stellate ganglia had been omitted, the adrenals accounted in part only for the pressor response to naloxone. For instance, in the two experiments of column 7 of Figure 1 in which evisceration had been omitted, the response was 20 and 31 mmHg, as compared to 0, 14, 15 and 25 mmHg in four other cats in which the adrenals had also been removed. And it was 15, 36 and 44 mmHg in the three experiments of column 9 in which removal of the stellate ganglia had been omitted, as compared to 6, 14, 22, 24 and 28 mmHg in five other cats in which the adrenals had been removed as well.

Effect of subcutaneous morphine

The adrenals play a minor role in the strong pressor response with tachycardia which occurs when 200 μg naloxone are injected intravenously 1 to 2 h after a subcutaneous injection of 1 to 2 mg/kg of morphine sulphate because a strong pressor response is also produced after removal of the adrenals. This is illustrated in Figure 4. The two arrows below record (a) indicate intravenous injections of 200 μg naloxone. The first injection was made 45 min after retroperitoneal removal of the adrenals. It produced no pressor

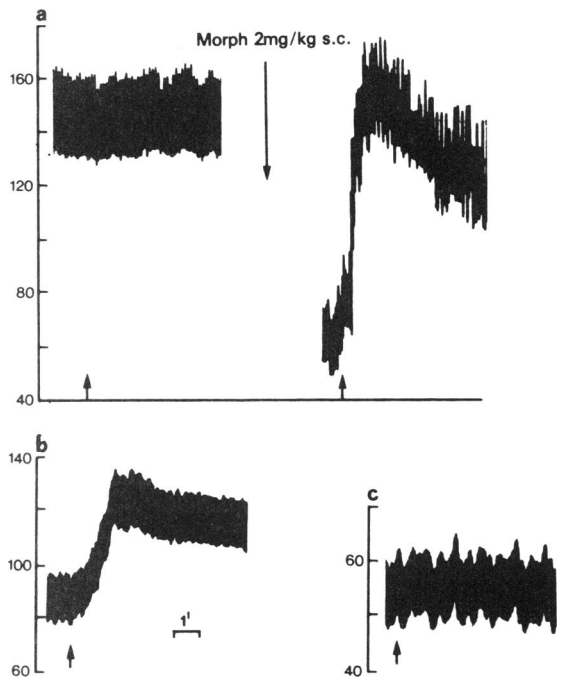

Figure 4 Arterial blood pressure from three cats (Cat No. 1, 2.6 kg, in (a); No. 2, 2.4 kg in (b), and No. 3, 3 kg in (c)) anaesthetized with chloralose and artificially ventilated. Short arrows (↑) indicate intravenous injections of 200 μg of naloxone. Record (a) from a cat (no. 1) with adrenals removed. During the interval and 90 min before the second naloxone injection, 2 mg/kg morphine sulphate injected subcutaneously. Records (b) and (c) from two eviscerated cats (nos 2 and 3) with stellate ganglia removed, vagi cut and sinus nerves tied; in cat no. 3 (c) adrenals removed as well. About 90 min before the intravenous injections of naloxone each cat received a subcutaneous injection of 2 mg/kg of morphine sulphate. Ordinates: arterial blood pressure in mmHg.

response which suggests that removal of the adrenals without other surgical procedures does not result in release of opioid peptides. The second injection was made 3 h after the first, and 2 h after a subcutaneous injection of 2 mg/kg of morphine sulphate. During these 2 h blood pressure had fallen gradually to about 60 mmHg. The injection of naloxone rapidly reversed this fall: this strong pressor response occurred in the absence of the adrenals.

The result is different when the cats are eviscerated, have their stellate ganglia removed, their vagi cut and their sinus nerves tied off. Then the adrenals are entirely responsible for the much smaller pressor response which is produced by naloxone when injected after the cat has received subcutaneous morphine. This is illustrated by Figure 4(b) and (c). These records were obtained from two eviscerated cats with stellate ganglia removed, vagi cut and sinus nerves tied off. The arrows again indicate intravenous injections of 200 μg naloxone made about 1 h after the cats had received a subcutaneous injection of 2 mg/kg of morphine sulphate. In (b), a pressor response occurred because the adrenals had not been removed; in (c) no pressor response occurred because the adrenals had been removed. The pressor response in (b) from the cat with the adrenals present resulted mainly from reversal of the effects of injected morphine and not of released opioid peptides, because preceding the morphine injection, three intravenous injections of naloxone had been made in this cat, and the pressor response had diminished with each injection, having become less than 20 mmHg with the third injection.

If the stellate ganglia had not been removed, or the cats had not been eviscerated, but the other three surgical procedures had been performed and morphine sulphate (2 mg/kg) was injected subcutaneously, a subsequent intravenous injection of 200 μg of naloxone produced a pressor response which was partly due to adrenaline secretion, because it was also obtained after removal of the suprarenals, though it appeared to be reduced in this condition.

Discussion

The fact that naloxone is a potent antagonist of morphine and of opioid peptides, but otherwise pharmacologically rather inert, is the basis for its use in detecting the release of opioid peptides. Whenever, in a particular experimental condition, naloxone has an action it would have acquired had morphine or opioid peptides been administered, it is probably because that particular experimental condition has resulted in a release of opioid peptides. Naloxone can thus be, and has been, used to provide indirect evidence for their release.

In the present experiments on anaesthetized cats, the action acquired was a pressor response with tachycardia which naloxone would also have had if the cat had been given an injection of morphine or of opioid peptides, and the particular condition responsible for the release of opioid peptides and therefore for the pressor response, was a number of certain surgical procedures. Though the evidence for the release of the opioid peptides obtained in this way is indirect, it would be difficult to interpret differently the rise in blood pressure with tachycardia obtained in the present experiments in response to naloxone.

The strongest pressor response indicating the greatest release of opioid peptides was obtained in cats that had undergone four surgical procedures, that is,

in cats that had been eviscerated, had their stellate ganglia removed, vagi cut and sinus nerves tied. In this condition the pressor response to naloxone resulted almost entirely from adrenaline secretion brought about by a sympathetic discharge, that is, by a central effect on the adrenal medulla. If either evisceration or removal of the stellate ganglia were omitted from the four surgical procedures, the somewhat smaller pressor responses then obtained with naloxone resulted from a sympathetic discharge, projecting not only to the adrenals, but in addition to the blood vessels and, if the stellate ganglia had remained *in situ*, to the heart as well. This means that the opioid peptides released by the various surgical procedures must have inhibited the sympathetic tone to blood vessels, heart and adrenals. Inhibition of sympathetic tone to blood vessels and heart has been shown to occur in anaesthetized cats when opioid peptides or morphine are injected (Feldberg & Wei, 1977, 1978a, b). The inhibition of sympathetic tone to the adrenal medulla was not known; it was revealed in the present experiments by the analysis of the pressor responses to naloxone, first for the released opioid peptides, but later for morphine as well.

The inhibitory effect of morphine on the sympathetic discharge to the adrenals occurs only in anaesthesia; in non-anaesthetized animals including cats, morphine has the opposite effect and produces a sympathetic discharge to the adrenals which results in hyperglycaemia. This effect as well as its abolition in anaesthesia has been known for a long time (for references see Feldberg & Shaligram, 1972). It is not known whether the exictatory action which initiates this sympathetic discharge is converted in anaesthesia into an inhibitory one, in which case the two actions would be on the same site and the same synapses, or if different sites and different synapses are affected by the two opposing central actions.

Since the pressor responses to naloxone are central effects, the effects of the released peptides must be central too; therefore the peptides need not be released into the blood stream. The results could equally well be explained if they were released into the cerebrospinal fluid, or if, after having acted at the site of release, they were at once destroyed before escaping into the blood or liquor. The released peptides could be either enkephalins (Hughes, *et al.*, 1975) or β-endorphin (Li & Chung, 1976; Bradbury *et al.*, 1976) and the stimulus for their release could be interruption of afferent sensory pathways from the viscera or the 'stress' associated with the surgical procedures.

Our results do not favour the view that the afferent impulses responsible for pain sensation release at the same time an enkephalin to counteract pain; they would suggest, rather, the opposite view: a continuous 'tonic' release of opioid peptides from interneurones in the afferent pathway which occurs in the absence of pain impulses and is inhibited by them, because interruption of afferent visceral pathways seemed to promote the release. If the release after the surgical procedures were brought about in this way, the released peptides would probably be enkephalins.

A different explanation in line with recent findings would attribute the release to the 'stress' associated with the surgical procedures. In that case the mediator would probably be β-endorphin because in rats, foot-shock-induced stress which promotes analgesia that is partly reversed by naloxone (Akil, Madden, Patrick & Barchas, 1976) has been shown to release this peptide from the pituitary and to increase its plasma level (Guillemin, Vargo, Rossier, Minik, Ling, Rivier & Bloom, 1977; Rossier, French, Rivier, Ling, Guillemin & Bloom, 1977). In these experiments, β-endorphin was determined by radio-immunoassay, but as pointed out by Smyth & Zakarian (1978), such measurements include in addition to β-endorphin, three related but pharmacologically rather inactive peptides, the acyl β-endorphin, C'-fragment and the acyl C'-fragment, which are normal constituents of brain and pituitary. Stress resulting in peptide release is thought to account also for the naloxone reversal of the endotoxin hypotension obtained in rats (Haladay & Faden, 1978), and in man, the hyperalgesic effect of naloxone in patients with post-operative pain, first described by Lassagna (1965), was attributed by Levine, Gordon, Jones & Fields (1978) to a reversal of the analgesic effect of opioid peptides released not by the physiological pain stimulus but by the stress associated with clinical pain. They demonstrated that following extraction of impacted wisdom teeth, naloxone caused increased pain and concluded that 'the prolonged duration and stress associated with clinical pain as opposed to experimental pain makes it particularly effective for activating the endogenous analgesic system'.

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