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C-FRAGMENT OF LIPOTROPIN— AN ENDOGENOUS POTENT ANALGESIC PEPTIDE

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1 A series of peptides derived from porcine lipotropin was examined for analgesic and other morphine-like properties on infusion into the cannulated third ventricle of cats.

2 Lipotropin (LPH 1-91) itself produced no analgesia or other morphine-like effects when infused in a dose of 150 μg.

3 C-fragment (LPH 61-91) produced strong long-lasting analgesia when infused in a dose of 10 or 20 μg; on a molar basis the potency was between 90 and 180 times that of morphine. The following morphine-like effects were also produced: shivering leading to fever, vasodilatation of the pinnae, mydriasis, opening of the palpebral fissures, tachypnoea with bouts of panting, vocalization, hyperexcitability, restlessness and catalepsy. All the effects, including analgesia, were abolished by an intraperitoneal injection of naloxone (1 mg/kg).

4 Hyperglycaemia, another central effect produced by morphine, was obtained with C-fragment infused in a dose of 60 μg.

5 On intravenous injection, C-fragment produced analgesia with a dose of about 200 μg/kg. Administered by this route, C-fragment was again more potent than morphine.

6 C′-fragment (LPH 61-87), LPH 61-78 and LPH 61-69, either had no analgesic effect or produced weak short-lasting analgesia when infused in doses up to 100 μg.

7 Methionine enkephalin (LPH 61-65) either produced very weak short-lasting analgesia or had no analgesic effect when infused in doses of between 30 and 400 μg.

8 *N*-methyl methionine enkephalin amide in which both termini of methionine enkephalin were protected against degradation by exopeptidases produced long-lasting analgesia when infused in doses of 150 to 180 μg; its analgesic potency was approximately 100 times less than that of C-fragment. Blocking only one terminus of methionine enkephalin did not appear to endow the peptide with analgesic properties. The *N*-methyl pentapeptide amide produced other morphine-like effects of which the most striking was catalepsy. All the effects were abolished by intraperitoneal naloxone (1 mg/kg).

Introduction

The C-fragment of lipotropin, recently also called β-endorphin (Li & Chung, 1976), was shown to exist as an endogenous polypeptide when it was isolated from porcine pituitary (Bradbury, Smyth & Snell, 1975). This 31 residue peptide was found to have high affinity for the opiate receptors in brain homogenates (Bradbury, Smyth, Snell, Birdsall & Hulme, 1976). In the present experiments which have been communicated to the Physiological Society (Feldberg & Smyth, 1976, 1977) it was shown in cats that the C-fragment is a strong analgesic, much stronger than morphine, that it has other central morphine-like actions and that all the effects are abolished by the morphine antagonist, naloxone. On the other hand, the pentapeptide methionine enkephalin, which forms one end of the peptide chain of C-fragment and which interacts with the opiate receptors in the guinea-pig ileum, mouse vas deferens and brain homogenates (for references see Hughes, Smith, Kosterlitz, Fothergill, Morgan & Morris, 1975) had at most a very weak and short-lasting analgesic action.

In addition to C-fragment and methionine enkephalin, three related peptides intermediate in length were examined for analgesic and other morphine-like properties. So were three synthetic peptides obtained by blocking methionine enkephalin at one or both termini; this was done to render the peptide resistant to enzymatic destruction because the relative ineffectiveness of methionine enkephalin as an analgesic might be due to its rapid degradation *in vivo*, a suggestion made by Hambrook, Morgan, Rance & Smith (1976).

Since the first communication of our results, the strong analgesic action of C-fragment has also been

obtained in rats (Graf, Szekely, Ronai, Dunai-Kovacs & Bajusz, 1976; Loh, Tseng, Wei & Li, 1976; Van Ree, de Wied, Bradbury, Hulme, Smyth & Snell, 1977; Bradbury, Smyth, Snell, Deakin & Wendlandt, 1977).

Methods

Cats of either sex weighing between 2.7 and 3.7 kg were used. The peptides and morphine were infused into the third ventricle caudal to the massa intermedia in a volume of 40 µl by a microinfusion pump which delivered this volume from a 100 µl syringe in 4 min 20 seconds. The infusions were made through a Collison cannula implanted into this part of the third ventricle in a preliminary aseptic operation under pentobarbitone sodium anaesthesia (36 mg/kg, i.p.) as described previously (Feldberg & Shaligram, 1972). After recovery from the operation the infusions were made without anaesthesia, the stilette being removed from the Collison cannula and a hollow stainless-steel needle (28 gauge) inserted instead. The length was such that when the needle passed through the rubber diaphragm of the cap and was fully inserted, it ended just beyond the end of the shaft of the cannula. The needle was connected by a length of fine Polythene tubing to the syringe of the microinfusion pump, needle and tubing being filled with the solution to be infused and the syringe with absolute alcohol.

Analgesia

To test for analgesia, the tail-pinch method of Russell & Tate (1975) was used in which a small diameter rod presses across the root of the tail. Graded pressure is applied to the tail for a 5 s period at about 1 min intervals. The pressure is exerted hydraulically and measured with a Bourdon gauge in kg/cm^2. Although this pressure is not identical with the force exerted on the tail it is proportional to it and is given without conversion as ordinates in the figures. The responses obtained with increasing pressure were classified as none, weak, medium or strong, according to the description given by Dey & Feldberg (1976). A weak response indicated that the cat noticed the pressure, but whether it felt pain was not certain. A medium response indicated definite pain and a strong response indicated intense pain. In the figures the signs 'none' and 'weak' are supplied with an interrupted vertical line. The length of the lines indicates the pressures which can apparently be exerted on the tail without eliciting pain and the increase in the height of the lines, the degree of analgesia obtained.

Catalepsy

To test for signs of catalepsy the cat was put in a nearly erect position with its forepaws placed over the upper rung of an inverted stool, or the cat was placed across two upper rungs of the stool. If cataleptic, the cat would retain these postures for several seconds or even for a few minutes without struggling, but its movements were not impaired. When climbing down the cat moved away, and when gently pushed from behind it jumped in a well co-ordinated manner. These simple procedures have previously been used to test for catalepsy produced in rabbits and cats by various drugs (Feldberg & Sherwood, 1954; 1955) including morphine (Banerjee, Burks, Feldberg & Goodrich, 1968; Feldberg & Shaligram, 1972; Dey & Feldberg, 1976).

Blood glucose

In one experiment blood glucose estimations were made. About 1.5 h before the first blood sample was withdrawn a nylon catheter was inserted, during a short-lasting Althesin anaesthesia, about 15 mm deep into the right femoral vein so that the opening was lying in the inferior vena cava, caudal to the entrance of the hepatic veins. The outer end of the catheter, closed by a rubber cap, was taken over to the outside of the thigh and held in position with adhesive tape. The patency of the catheter was ensured by flushing it with an injection of 1 ml of 0.9% w/v NaCl solution (saline) containing 50 u heparin through the cap after each collection of a blood sample. Further, to prevent the blood samples from being contaminated with the saline-heparin solution or blood stagnating in the catheter, 0.75 ml of blood was withdrawn immediately before a blood sample was taken, and discarded. To prevent coagulation, the blood samples (2 ml) were collected in ammonium fluoride and ammonium oxalate as described by Anderson (1969). Glucose concentration was determined with the automated glucose oxidase method described by the Sigma Technical Bulletin No. 970 (1970).

Peptides and drugs

For lipotropin (LPH) and the peptides which form part of its peptide chain the number of amino acids and their position in the chain are given together with the molecular weight (mol. wt.). Porcine lipotropin (LPH 1–91) which has a mol. wt. of 9894 was isolated from porcine pituitary gland according to the method of Li, Barnafi, Chretien & Chung (1965) as modified by Bradbury *et al.* (1975). C-fragment (LPH 61–91, mol. wt. 3423.7) and C′-fragment (LPH 61–87, mol. wt. 2982.3) were isolated by extraction of porcine pituitary glands and obtained in homogeneous form by ion exchange chromatography (Bradbury, Smyth & Snell, 1975; 1976). About 30 mg of C-fragment and 10 mg of C′-fragment were obtained from 1200 pituitary glands. The octadecylpeptide LPH 61–78 (mol. wt. 2006.1) was obtained by mild

chymotrypsin digestion of C-fragment (1:500) for 1 h at 37°C; its isolation was by chromatography on a column (40 x 1 cm) of SP25 with a gradient from 0–1M sodium chloride as eluent at pH 7.0 (Massey & Smyth, unpublished experiments). The nonapeptide LPH 61–69 (mol. wt. 1019.2) and the pentapeptide methionine enkephalin (LPH 61–65, mol. wt. 573.7) were synthesized by Bradbury & Smyth (unpublished experiments) using the solid phase method of Merrifield (1963). *N*-methyl methionine enkephalin (mol. wt. 587.8), methionine enkephalin amide (mol. wt. 572.8) and *N*-methyl methionine enkephalin amide (mol. wt. 586.8) were prepared by Bradbury, Smyth & Snell (unpublished experiments). The morphine preparation used was morphine sulphate B.P. (MacFarlane Smith Ltd, Edinburgh). Its mol. wt. is 758.8 but the salt contains two molecules of morphine. Therefore, for comparing its activity with those of the peptides on a molar basis, half the mol. wt. was taken. Naloxone hydrochloride (Endo Laboratories, New York) was used.

Results

Lipotropin (LPH 1–91)

Infused into the third ventricle in a dose of 150 μg lipotropin produced no analgesia or other morphine-like effects.

C-fragment (LPH 61–91)

Infused into the third ventricle in a dose of 10 or 20 μg C-fragment produced strong long-lasting analgesia. In cats in which the effect was compared with that of morphine, analgesia was as strong though not as long lasting as that produced by 100 or 200 μg of morphine sulphate similarly infused. On a molar basis the analgesic potency of C-fragment was between 90 and 180 times that of morphine.

The analgesia produced by 10 μg of C-fragment is illustrated in Figure 1a. It developed within a few minutes, reached its maximum in about 30 min and after about 1.5 h began to subside slowly. Some analgesia was still present 1 h later, i.e. about 2.5 h after the infusion. In this cat the analgesia which developed following the infusion of 200 μg of morphine sulphate was stronger and longer-lasting than that following the infusion of 10 μg of C-fragment. This is shown in Figure 1b which is a record obtained from the same cat nine days later. No analgesia developed on infusion of 2.5 μg of C-fragment.

On intravenous administration through the cephalic vein of a foreleg, C-fragment was less potent; yet it was again more potent than morphine. In the experiment of Figure 2, 0.57 mg of C-fragment injected intravenously into a 3 kg cat produced definite analgesia lasting about 20 min, whereas 1 mg of morphine sulphate similarly injected had no analgesic effect. In another cat weighing 2.5 kg, an intravenous injection of 0.62 mg of C-fragment produced analgesia of approximately the same intensity lasting about 30 minutes.

The infusion into the third ventricle of 10 or 20 μg of C-fragment produced other effects which are obtained when morphine is introduced into the liquor space (Feldberg & Shaligram, 1972) such as shivering resulting in fever, vasodilatation in the pinnae, tachypnoea with bouts of panting, mydriasis, widening of the palpebral fissures, vocalisation, hyper-excitability with restlessness and signs of catalepsy. Of these effects, shivering, vasodilatation of the pinnae and tachypnoea with panting occurred also when C-fragment was infused in a dose (2.5 μg) sub-threshold for analgesia.

Shivering was the first effect to appear. It began during the infusion of C-fragment, quickly became vigorous and continued for periods varying between 20 and 90 minutes. Figure 3 shows the rise in rectal temperature resulting from such shivering following the infusion of 10 μg of C-fragment.

Vasodilatation of the pinnae and tachypnoea began a few minutes after the infusion. Vasodilatation disappeared after 20 to 90 minutes. Tachypnoea developed rapidly into bouts of panting which continued for up to 2 hours. The time course for mydriasis, opening of the palpebral fissures and vocalization was about the same. The pupils became maximally dilated and the eyes opened wide. In this condition the cats with their ears pricked would stare vacantly without blinking for long periods. Vocalization consisted of bouts of miaowing which occurred at shorter and shorter intervals and with increasing duration until it became continuous, the miaowing becoming louder and more frequent.

Hyperexcitability and restlessness developed later than the other effects, occurring 30 min or more after the infusion. During the state of hyperexcitability the cat, when lightly touched, made rapid, brisk movements usually leading to circling in the relatively small cage. Later on, the movements leading to circling occurred spontaneously. Signs of catalepsy were observed in a few experiments, 1–1.5 h after the infusion. When placed in a nearly erect position with their forepaws over the upper rung of an inverted stool, the cats retained the posture for 30 s or longer.

C-fragment in large doses, like morphine, was found to be strongly hyperglycaemic, as illustrated by the experiment of Figure 4. The blood glucose concentration rose from 60.0 and 63.5 before, to 280 mg/100 ml 1.5 h after the infusion of 60 μg of C-fragment, whilst strong analgesia developed. When tested after the fourth and fifth blood sample the cat did not react at all to the maximum pressure of 4 kg/cm² exerted on

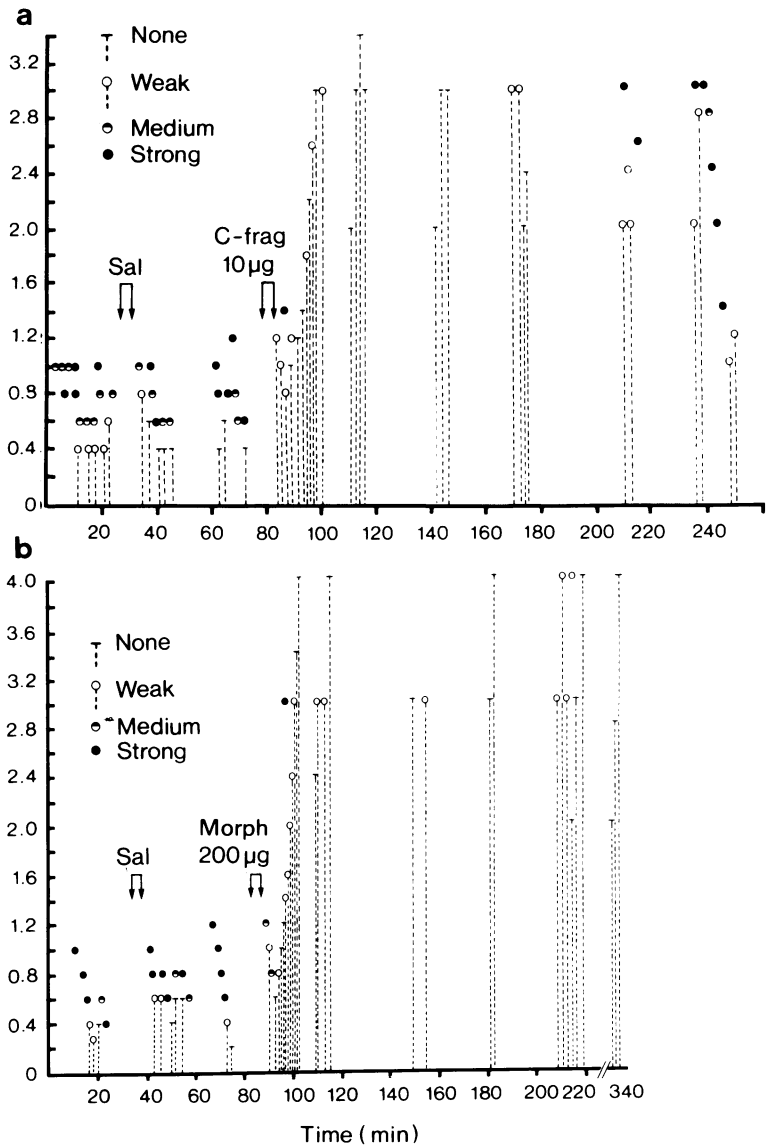

Figure 1 Analgesia produced in a cat (3.2 kg) (a) on infusion of 10 µg of C-fragment (C-Frag) and (b) of 200 µg of morphine sulphate (Morph) into the third ventricle; (b) was obtained 9 days after (a). Sal = control infusion of 40 µl saline. In this figure as well as in Figures 2, 5, 6 and 7, the ordinates give the pressure in kg/cm² exerted for 5 s on the root of the tail, and the degree of analgesia is indicated by the rise in the interrupted lines.

the tail. In Figure 4 the hyperglycaemic effect is compared with that previously obtained (Feldberg & Shaligram, 1972) with 750 µg of morphine sulphate injected into a lateral ventricle. The three curves give the mean, the maximal and the minimal results obtained in nine cats.

Intermediate LPH fragments

LPH 61–87, 61–78 and 61–69, either had no analgesic effect or produced short-lasting weak analgesia when infused in doses up to 100 µg. Reducing the peptide chain of C-fragment by only

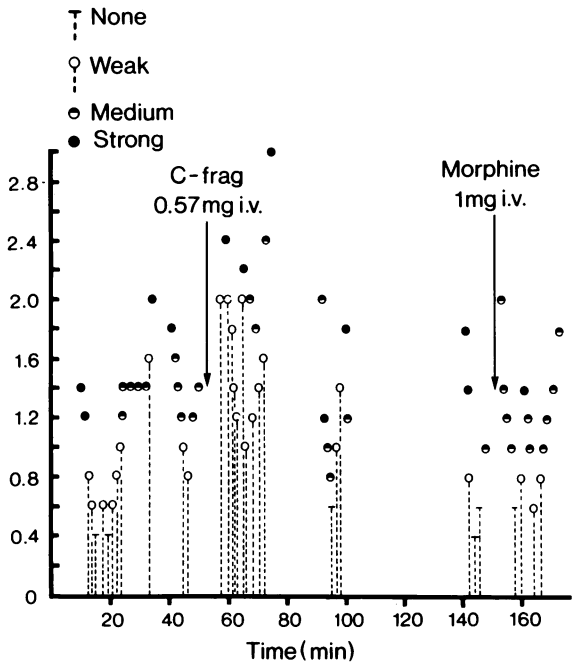

Figure 2 Analgesia produced in a cat (3 kg) on intravenous injection of 0.57 mg of C-fragment (C-Frag). Comparison with intravenous injection of 1 mg of morphine sulphate.

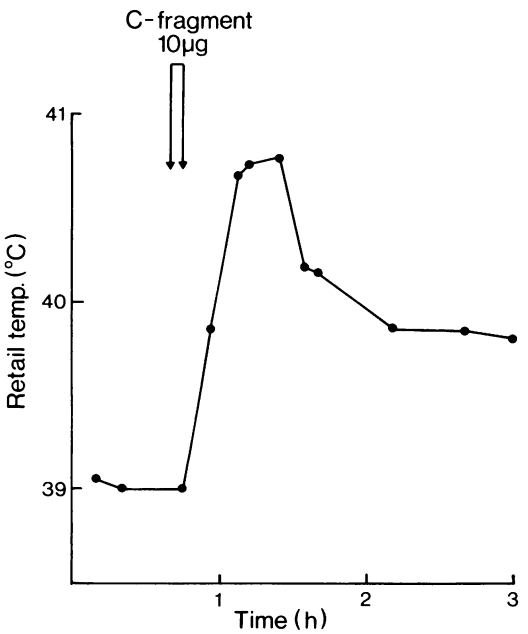

Figure 3 Fever produced in a cat (3.0 kg) on infusion of 10 µg of C-fragment into the third ventricle. Ordinates: rectal temperature in °C.

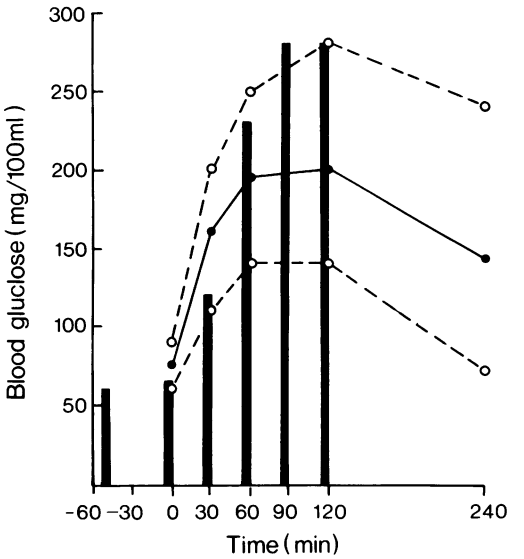

Figure 4 Hyperglycaemia produced in a cat (3.0 kg) on infusion of 60 µg of C-fragment into the third ventricle. Ordinates are mg glucose in 100 ml blood. The vertical columns give the times when venous blood samples were taken before and after the infusion and the height of the columns their glucose concentration. The three curves are plotted from experiments of Feldberg & Shaligram (1972) and give for comparison the maximal, mean and minimal hyperglycaemic effects produced in nine cats on infusion of 750 µg of morphine sulphate into a lateral ventricle.

four amino acids resulted in a loss of its analgesic property because the C'-fragment (LPH 61–87) infused in doses of 30 to 100 µg produced no analgesia or other morphine-like effects. Larger doses were not tested.

LPH 61–78 was infused in one cat first in a dose of 50 and later of 100 µg. Both infusions produced some shivering and the infusion of the larger dose was followed by weak analgesia which lasted less than 20 min. The infusion of the nonapeptide LPH 61–69 in a dose of 100 µg had no analgesic effect, but it produced some shivering and vasodilatation in the pinnae.

Methionine enkephalin (LPH 61–65)

The pentapeptide had at most a very weak and short-lasting analgesic effect when infused in doses of between 30 and 200 µg. In some of the experiments the pressure that could be exerted on the tail without producing pain responses was a little stronger during the first few minutes following the infusion than before it, suggesting a weak short-lasting analgesia. However,

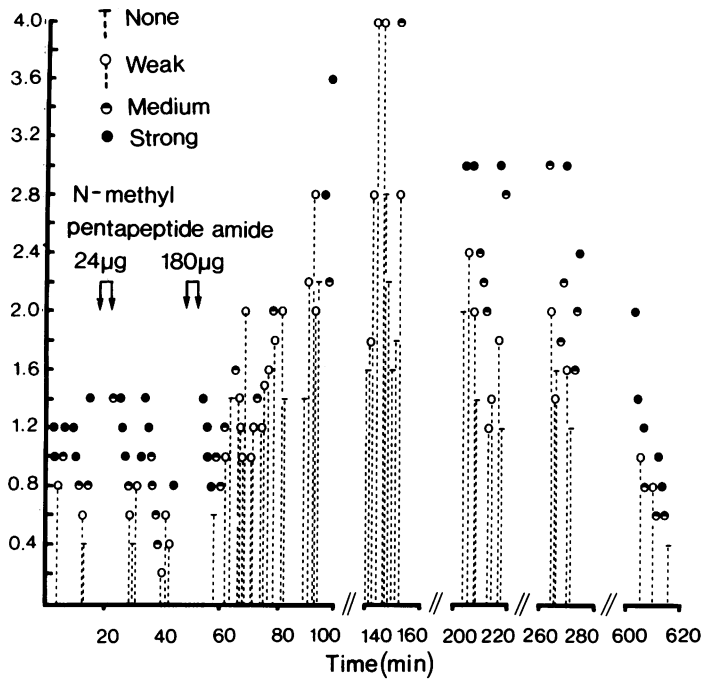

Figure 5 Analgesia produced in a cat (2.8 kg) on infusion of 180 µg *N*-methyl methionine enkephalin amide into the third ventricle. No analgesia on infusion of 24 µg.

the effect was not dose-dependent; in one experiment it did not occur on infusion of 400 µg. On the other hand weak, short-lasting analgesia was sometimes obtained following control infusions of saline. With the larger doses of methionine enkephalin some shivering, tachypnoea and in one experiment panting were obtained.

N-methyl methionine enkephalin amide

The pentapeptide amide produced strong analgesia when infused into the third ventricle in doses of 150 to 180 µg. It was less potent than C-fragment since it was ineffective in doses of 10 to 30 µg. The effect of 24 and 180 µg is shown in Figure 5. After 24 µg no analgesia developed; after 180 µg analgesia developed within a few minutes, gradually increased and became maximal in about 1.5 hours. It then gradually diminished but had not fully subsided 3.5 h after the infusion. On a molar basis the analgesic potency of the *N*-methyl methionine enkephalin would appear to be about one hundredth that of C-fragment since the effect of 150 to 180 µg corresponded to that of 10 µg of C-fragment.

The infusion of 150 to 180 µg of the *N*-methyl methionine enkephalin amide produced in addition to analgesia, shivering and vasodilatation of the ear vessels as well as some mydriasis and tachypnoea, but less pronounced than following the infusion of C-fragment. Mydriasis did not become maximal and the rate of respiration did not exceed 80/minute. The most striking effect, apart from analgesia was the deep stupor and catalepsy which developed within 15 min of the infusion, became maximal during the first hour and then gradually subsided during the next hour although analgesia continued for some time. During the condition of deep catalepsy the cat tended to lie on its side and made no effort to get up when touched or handled. When put into a nearly erect position with its forepaws over the upper rung of an inverted stool, or when placed horizontally across the two rungs, the cat did not resist and retained these postures for a few minutes; but when it subsequently moved away it showed no signs of ataxia. The condition of catalepsy was followed by one in which the cat became extremely affectionate, a condition also seen after morphine infusion. Finally about 4 h after the infusion the cat became very sleepy.

Both ends of the methionine enkephalin had to be blocked for the pentapeptide to become an analgesic substance. Blocking the C-terminus with an amide group did not endow the pentapeptide with analgesic or other morphine-like properties. Infused in a dose of 200 µg, methionine enkephalin amide produced no

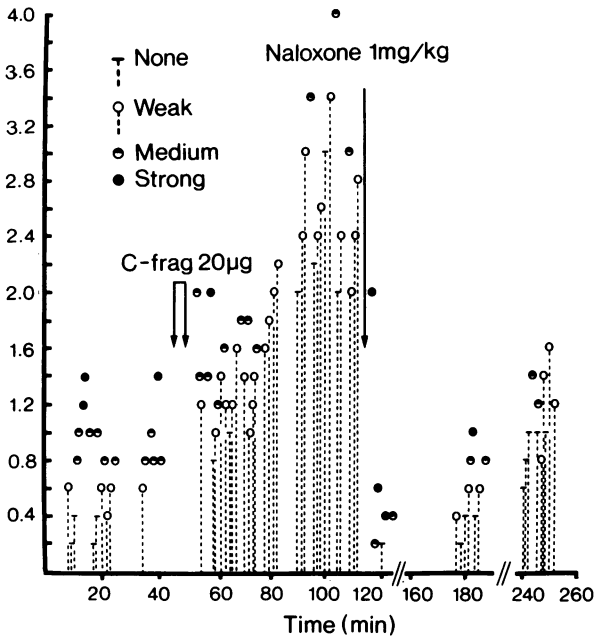

Figure 6 Analgesia produced in a cat (2.8 kg) on infusion of 20 µg of C-fragment (C-frag) into the third ventricle; reversal by naloxone (1 mg/kg i.p.).

analgesia; the only effect observed was some shivering. Blocking the other end of methionine enkephalin with an *N*-methyl group produced a pentapeptide of relatively low solubility which could be infused only in a dose of 70 µg. This dose produced no analgesia, shivering or other morphine-like effect.

Naloxone

Analgesia as well as the other morphine-like effects produced on infusion of morphine sulphate, C-fragment and *N*-methyl methionine enkephalin amide were abolished within a few minutes of an intraperitoneal injection of naloxone 1 mg/kg.

The effect of naloxone on the analgesia produced by the infusion of 20 µg of C-fragment is illustrated in Figure 6. Within 3 min of the naloxone injection, analgesia had disappeared. In this experiment the cat became even more sensitive to the pressure exerted on the tail during the first 10 min following the naloxone injection. The experiment illustrates another feature, the return of the analgesic effect as the naloxone effect subsided. Before the naloxone injection the pupils were dilated, the cat was stuporous, staring with wide open eyes, rarely blinking, and there were definite signs of catalepsy. Within the first 3 min of the injection, mydriasis began to disappear, the eyes were no longer wide open, blinking occurred frequently and the cat became lively and alert. Later the pupils became slit-

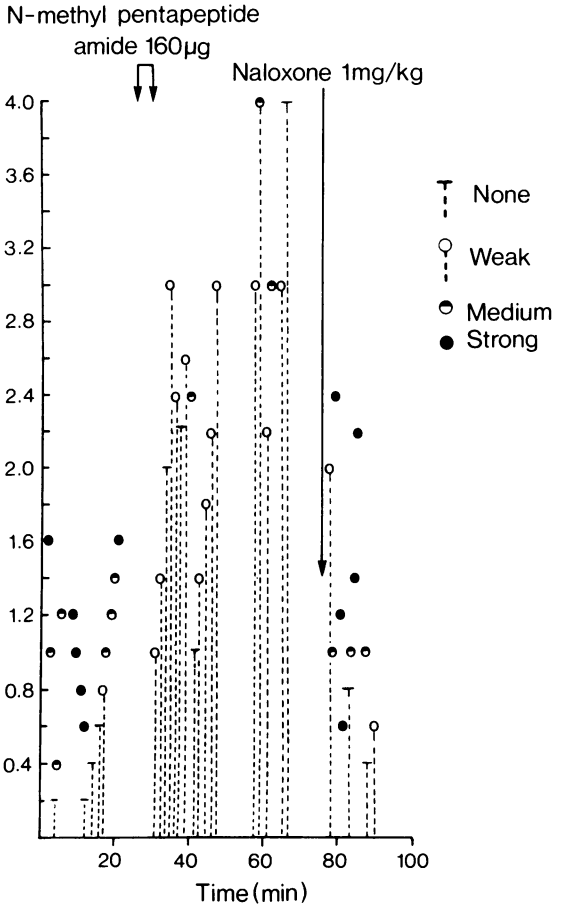

Figure 7 Analgesia produced in a cat (2.7 kg) on infusion of 160 µg of *N*-methyl methionine enkephalin amide into the third ventricle; reversal by naloxone (1 mg/kg i.p.).

like and all signs of catalepsy were found to have disappeared.

The effect of naloxone on the analgesia produced by the infusion of 160 µg of the *N*-methyl methionine enkephalin amide is illustrated in Figure 7. Again analgesia disappeared within the first three minutes. Before the naloxone injection the cat was shivering, stuporous, lying on its side and deeply cataleptic. It was striking that within 2 min of the injection, shivering stopped, stupor disappeared, the cat got up, became lively and alert and when tested 4 min after the injection, no longer showed any signs of catalepsy.

Discussion

The main result of the present experiments is the strong analgesia produced by C-fragment and the

reversal of this effect by the morphine antagonist naloxone. The analgesic action of C-fragment was found to be stronger than that of morphine whether administered by infusion into the third ventricle or injection into the cephalic vein. On infusion the potency of C-fragment was between 90 and 180 times greater than that of morphine. A closer comparison was not attempted because relatively small amounts of C-fragment were available and the number of experiments was limited.

Although the main clinical use of morphine is based on its analgesic property, morphine produces other central effects which vary in different species. Those produced in cats were also obtained with C-fragment; one of them was hyperglycaemia. For this effect larger doses both of morphine and of C-fragment are required than those necessary to produce analgesia. As a hyperglycaemic agent, too, C-fragment appeared to be at least 100 times more potent than morphine, though it has to be pointed out that the methods used for comparing the potency of the two substances were slightly different, C-fragment being infused into the third ventricle whereas morphine was injected into a lateral ventricle.

Recently it was shown (Belluzi, Grant, Garsky, Sarantakis, Wise & Stein, 1976) that the injection of methionine enkephalin into the cerebral ventricles of rats results in a short-lasting weak analgesia. In our experiments with infusion into the third ventricle of cats, no definite evidence for such an action was obtained. It may be that with our relatively slow method of infusion, a weak short-lasting analgesic effect would not be revealed. Alternatively methionine enkephalin might simply be inactivated more rapidly by the peptidases in the cat than in the rat.

If the ineffectiveness of methionine enkephalin as an analgesic is due to its rapid destruction by exopeptidases *in vivo* as suggested by Hambrook *et al.* (1976), making it more resistant to these enzymes should produce an analgesic peptide. To achieve this the two termini of the pentapeptide were blocked, one by an *N*-methyl, the other by a *C*-amide group. The resulting *N*-methyl pentapeptide amide was found to produce long-lasting analgesia when infused into the third ventricle; but its potency as an analgesic was less than one hundredth that of C-fragment. This result resembles that obtained by Pert, Pert, Chang & Fong (1976) who substituted glycine at position 2 of methionine enkephalin by the D-isomer of alanine and found that the modified enkephalin gave long-lasting analgesia on injection into the cerebral ventricles of rats. Again it was less potent than morphine and therefore much less potent than C-fragment. The *N*-methyl enkephalin amide also produced other morphine-like effects. Catalepsy was particularly striking and was more pronounced than that obtained with C-fragment, suggesting that the relative potencies of different morphine-like effects may vary with different analgesic peptides.

Methionine enkephalin which forms one end of the peptide chain of C-fragment, cannot be solely responsible for its strong analgesic action because the C′-fragment which lacks only four amino acids at the C-terminus exerted no analgesic or other morphine-like effect when infused in doses much greater than those effective with C-fragment.

When considering the possible physiological roles of C-fragment and methionine enkephalin in reducing pain sensation in the body, the fact has to be taken into account that C-fragment is not only a much stronger analgesic than methionine enkephalin but is by far the most potent analgesic peptide known to occur endogenously. It is also a stronger analgesic than any of the synthetic pentapeptides obtained by modifying the enkephalin molecule so as to render it less liable to enzymatic destruction *in vivo*. There are in principle three possibilities of how a naturally occurring analgesic peptide might exert its analgesic function.

First, it may reach the nervous structures in the brain via the blood stream by being released into the circulation. This possibility can probably be excluded for methionine enkephalin because of its rapid destruction by enzymes in blood (Hambrook *et al.* 1976); but it has to be considered for C-fragment. Not only is C-fragment much more stable than methionine enkephalin to degradation by enzymes present in brain homogenates (Austen & Smyth, unpublished experiments) but it is also effective on intravenous injection. Although about 30 to 60 times larger doses were required than on infusion into the third ventricle, this is the usual difference in efficacy with these two routes of administration for drugs acting on structures lining the ventricular walls or the ventral surfaces of the brain stem.

Second, the peptide may reach the nervous structures via the liquor space by being released into the cerebral ventricles or into the subarachnoid space. For methionine enkephalin this possibility can probably also be excluded because of its relative ineffectiveness in producing analgesia when infused into the third ventricle. On the other hand, C-fragment produces strong analgesia when applied by the intraventricular route. If C-fragment were to be released from the pituitary into the liquor space one would expect it to be released not into the cerebral ventricles but into the subarachnoid space beneath the ventral surface of the brain stem and it would then have to exert its analgesic effect from this site. This should create no difficulty. Morphine for instance, infused into the subarachnoid space beneath the ventral surface of the brain stem produces analgesia. In fact, its analgesic effect on injection into the cerebral ventricles is thought to be due, at least in part, to an action on structures at the ventral surface of the brain stem after having passed from the ventricles into the subarachnoid space (Dey & Feldberg, 1976).

Third, the analgesic peptides could be released

during neuronal activity perhaps as transmitter substances from nerve endings and act locally at the site of release. This has been considered as a mechanism by which methionine enkephalin might produce its analgesic effect (Smith, Hughes, Kosterlitz & Sosa, 1976). Its rapid destruction *in vivo* might in fact be an advantage for such a transmitter function, but there is no experimental evidence for a transmitter function as yet. Such a mechanism could not be envisaged for the C-fragment released from the pituitary but the C-fragment occurs also in brain tissue (Bradbury, Feldberg, Smyth & Snell, 1976). For the extra-pituitary C-fragment a transmitter function can be envisaged even though the known transmitters are relatively small molecules. As a transmitter for its analgesic and other morphine-like effects the action of C-fragment would not necessarily be evanescent.

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