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INHIBITORY ACTIONS OF METHIONINE-ENKEPHALIN AND MORPHINE ON THE CAT CAROTID CHEMORECEPTORS

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1 The effects of intracarotid injections of methionine-enkephalin (Met-enkephalin) and morphine on chemoreceptor activity recorded from the peripheral end of a sectioned carotid sinus nerve have been studied in cats anaesthetized with pentobarbitone.

2 Met-enkephalin caused a rapid, powerful, inhibition of spontaneous chemoreceptor discharge, the intensity and duration of which was dose-dependent.

3 Morphine was a less potent inhibitor of spontaneous chemoreceptor discharge, and the inhibition it evoked was rather variable and tended to be biphasic. Low doses of morphine caused a slight increase in discharge.

4 Naloxone (0.2 mg i.c.) slightly increased spontaneous discharge, greatly reduced the chemo-inhibition caused by morphine, and reduced the inhibitory effect of Met-enkephalin. A higher dose of naloxone (0.8 mg) caused a substantial reduction of the Met-enkephalin effect.

5 Chemo-excitation evoked by intracarotid injections of acetylcholine, CO_2-saturated Locke solution, and sodium cyanide were only slightly and somewhat variably reduced following injections of Met-enkephalin, whereas the inhibitory effect of dopamine was potentiated. Following morphine administration, responses to acetylcholine and sodium cyanide were reduced slightly, whereas those to CO_2 and dopamine were potentiated.

6 Responses to acetylcholine and CO_2 were slightly potentiated during infusion of Met-enkephalin (50 μg/min, i.c.) and the response to sodium cyanide was slightly reduced.

7 It is concluded that naloxone-sensitive opiate receptors are present in the cat carotid body; when activated they cause inhibition of spontaneous chemoreceptor discharge. The physiological role of these receptors and the identity of any endogenous ligand remains to be established.

Introduction

Methionine-enkephalin (Met-enkephalin) is a potent inhibitor of spontaneous chemoreceptor discharge in the cat (McQueen, 1979), and the present neuropharmacological study was undertaken to investigate further this action of Met-enkephalin. It was also considered of interest to determine whether morphine has the same effect as Met-enkephalin on the cat carotid chemoreceptors.

Methods

Experiments were performed on ten cats weighing between 2.1 and 3.5 kg, median weight 2.9 kg. They were anaesthetized with pentobarbitone sodium (42 mg/kg i.p. initially, supplemented by i.v. administration of 10% of the initial dose every 1 to 2 h), artificially ventilated and paralysed by gallamine triethiodide (3 mg/kg i.v.). Full details of the experimental techniques have been given previously (McQueen, 1977; Docherty & McQueen, 1978).

Electrical activity of chemoreceptor units (1 to 5 units) was recorded from filaments of the peripheral end of a sectioned sinus nerve, passed through a pulse height (window) discriminator, and quantified with the aid of a PDP-8 computer. The ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in modified Locke solution (McQueen, 1977). Drug solutions (0.1 ml) were injected into the common carotid artery ipsilateral to the sinus nerve from which activity was being recorded, and washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2: 95% air in a water bath at 37°C; injections were made over 2 s. The catheter was introduced into the common carotid artery via the lingual artery and advanced until its tip lay about 2 cm caudal to the carotid bifurcation. In

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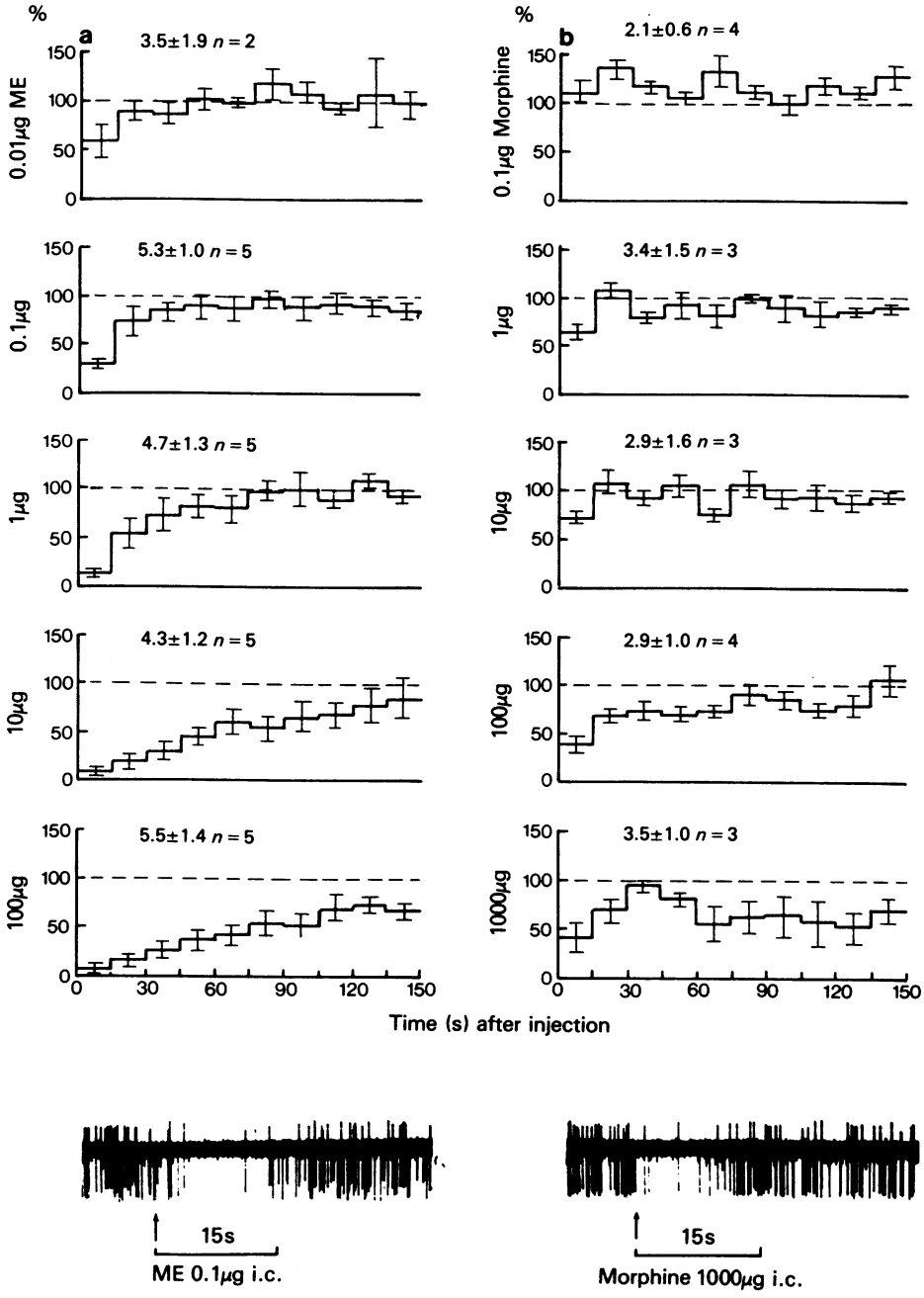

Figure 1 The upper part illustrates the effects of various doses of methionine-enkephalin (ME) (a) and morphine (b) on spontaneous chemoreceptor discharge. Discharge was averaged over 15 s periods following the injection and expressed as a percentage of the averaged discharge in the 15 s pre-injection control period. Data from *n* experiments were pooled and are shown as the mean percentages; vertical lines show s.e. mean. Averaged values (ct/s) ±s.e. mean for the control (100%) periods are given.

The neurograms in the lower part of the figure, taken from one experiment, show the early part of the somewhat similar inhibition of chemoreceptor discharge caused by injecting (arrow) (a) ME 0.1 μg i.c. and (b) morphine 1000 μg i.c.

some experiments a second catheter was positioned in the common carotid artery, this time via the superior thyroid artery, and used for the infusion of drug solutions (0.5 ml/min for 65 s; Braun, Unita).

Drugs used were: morphine sulphate, gallamine triethiodide (May & Baker); sodium cyanide, acetylcholine iodide (B.D.H.); methionine-enkephalin (Uniscience); dopamine hydrochloride (Koch Light) and naloxone hydrochloride (Endo), kindly given to us by Professor W. Feldberg.

Results

Effect of methionine-enkephalin injections on spontaneous chemoreceptor discharge

Met-enkephalin caused a dose-dependent reduction in spontaneous chemoreceptor discharge with a rapid onset, starting within 1 to 2 s of beginning the injection. During the early part of the response there was total inhibition of chemoreceptor discharge, then spontaneous activity returned gradually, reaching control (pre-injection) levels within 30 to 45 s following low doses of Met-enkephalin, but taking up to 5 min to recover after high doses (see Figure 1a). The inhibitory effect was consistent, as can be gauged from the standard errors, and there was no evidence of tachyphylaxis occurring when doses were administered at 7 min intervals. The effect of Met-enkephalin seemed to be potentiated slightly after morphine had been injected. Intravenous injections of Met-enkephalin (10 to 100 μg) also inhibited chemoreceptor discharge, although to a lesser extent and after a longer delay than the same doses by intracarotid injection.

Low doses of Met-enkephalin had little or no effect on blood pressure (BP), whereas higher doses (i.c. or i.v.) caused a fall in BP (see Figure 2).

Effects of morphine injections on spontaneous chemoreceptor discharge

The over-all effect of morphine on spontaneous chemoreceptor discharge was inhibitory, although the lowest dose studied, 0.1 μg, caused a slight increase in discharge (see Figure 1b). Higher doses were associated with an inhibition of discharge which began within 1 to 2 s of starting the injection (Figure 1b) and was dose-related. The initial inhibition lasted for 15 to 45 s after which discharge returned towards pre-injection control levels. There was then a delayed or secondary inhibition of spontaneous discharge, an effect which was most clearly seen after the highest dose of morphine (see Figures 1b, 3a).

Responses to morphine were rather variable, as can be seen from the standard errors, but there was no evidence of tachyphylaxis, and responses to a low dose of morphine injected before and after the highest dose were very similar. The higher doses of morphine caused a fall in BP (see Figure 2).

Effects of naloxone

In an adequately anaesthetized cat, which had received neither Met-enkephalin nor morphine, naloxone (0.2 mg i.c.) caused a slight increase in chemoreceptor discharge and, after a delay of 15 to 45 s, a rise in BP (Figure 2) lasting for at least 30 min. Additional doses of naloxone (0.4, 0.8 and 1.6 mg i.c. at 7 min intervals) had no further effect on chemoreceptor discharge or BP.

When naloxone (0.2 mg i.c.) was injected during experiments in which Met-enkephalin and/or morphine had previously been administered, there was also an increase, albeit somewhat variable, in spontaneous chemoreceptor discharge (Figure 2) and a rise in BP.

Comparison of responses to Met-enkephalin and to morphine obtained before and after injecting naloxone showed that, apart from a slight initial inhibition, the inhibitory action of morphine was virtually abolished, and there was evidence of an over-all increase in discharge. The chemo-inhibitory effect of Met-enkephalin was much reduced (see Figures 2 and 3).

Dose-response data were obtained by expressing the number of impulses in the post-injection period as a percentage of the number of impulses which would have been likely to occur in the same period had the pre-injection control discharge (averaged over 15 to 30 s) continued unaltered, and plotting this value against $\log_{10}$ dose. It can be seen from Figure 3 that naloxone shifts the Met-enkephalin dose-response line to the right, both for 60 s and 150 s post-injection periods. After intracarotid injection of naloxone (0.2 mg), morphine tended to increase discharge over these periods, this effect being inversely related to dose.

A higher dose of naloxone (0.8 mg i.c.) completely abolished the inhibitory action of morphine (1 mg i.c.) and greatly reduced the inhibitory reponse to Met-enkephalin; over-all there was an increase in discharge following lower doses of Met-enkephalin (see Figure 4).

Although naloxone reduced the hypotensive action of Met-enkephalin and morphine, slightly after 0.2 mg i.c. (see Figure 2) and to a greater extent after 0.8 mg i.c., falls in BP were still obtained even when the chemo-inhibitory response had been abolished.

Evoked responses

Injections (i.c.) of acetylcholine (ACh, 50 μg), carbon dioxide-saturated Locke solution (CO_2, 0.3 ml), sodium cyanide (NaCN, 5 μg) and dopamine (5 μg)

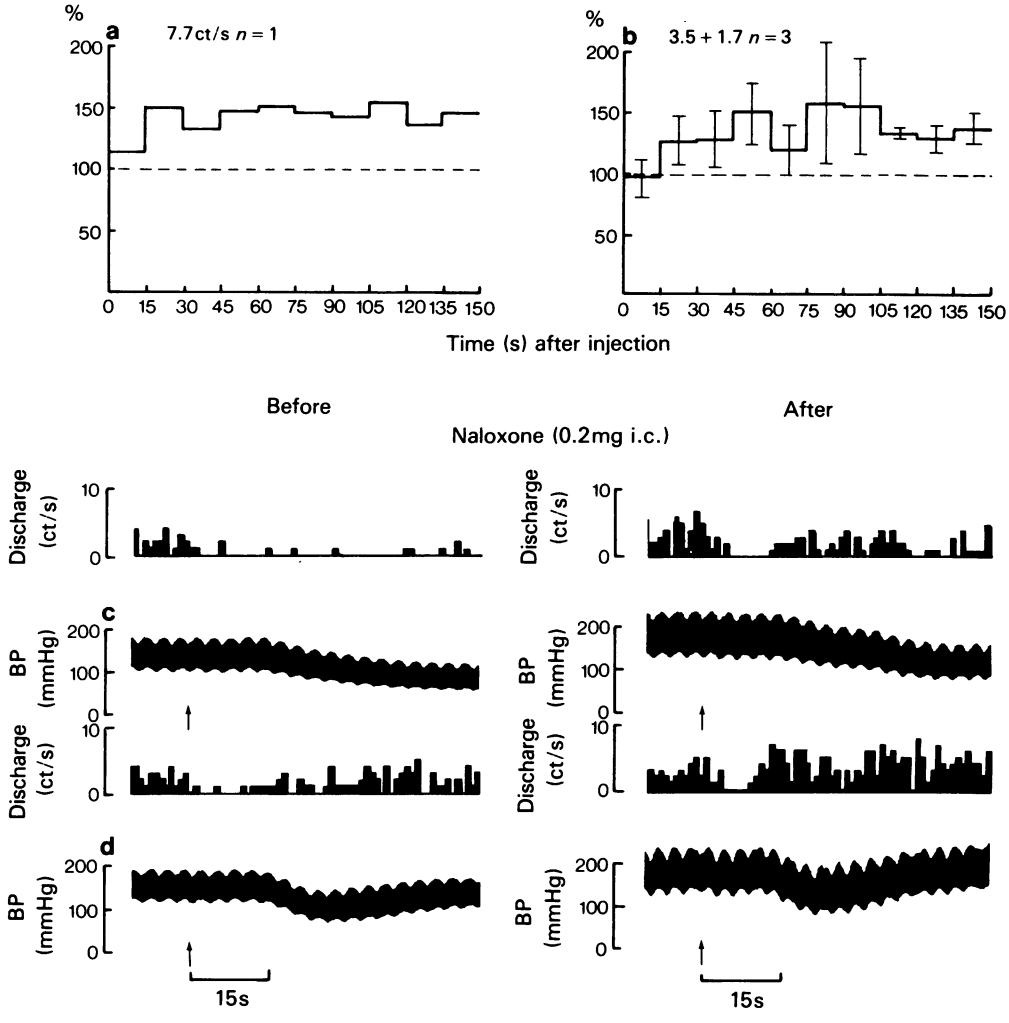

Figure 2 In (a) the effect of naloxone (0.2 mg i.c.) on spontaneous chemoreceptor discharge in an experiment during which no methionine-enkephalin (Met-enkephalin) or morphine had been administered is illustrated; (b) is the averaged spontaneous chemoreceptor discharge from three experiments in which the same dose of naloxone (0.2 mg i.c.) was injected following prior administration of Met-enkephalin and/or morphine. Details as for Figure 1.

Met-enkephalin 100 μg i.c. (c) and morphine 1000 μg i.c. (d) were injected, at the arrows, before and after naloxone (0.2 mg i.c.). Their effects on chemoreceptor discharge and BP are shown, and it can be seen that chemoreceptor inhibition was greatly reduced by naloxone, whereas the hypotension was less affected by this dose of naloxone.

were made before and 5 to 20 min after a series of injections of either Met-enkephalin or morphine. The results obtained (Table 1) showed that the stimulant action of ACh, CO_2, and NaCN were slightly and somewhat variably reduced after Met-enkephalin, whereas the inhibitory effect of dopamine was potentiated. Following morphine administration, responses to ACh and NaCN were reduced slightly, whereas those to CO_2 and dopamine were potentiated.

Responses were also obtained before and during an infusion of Met-enkephalin (50 μg/min i.c.), this dose being sufficient to inhibit spontaneous discharge throughout the infusion period. The effects of ACh and CO_2 were slightly potentiated whereas the response to NaCN was slightly reduced (see Table 1).

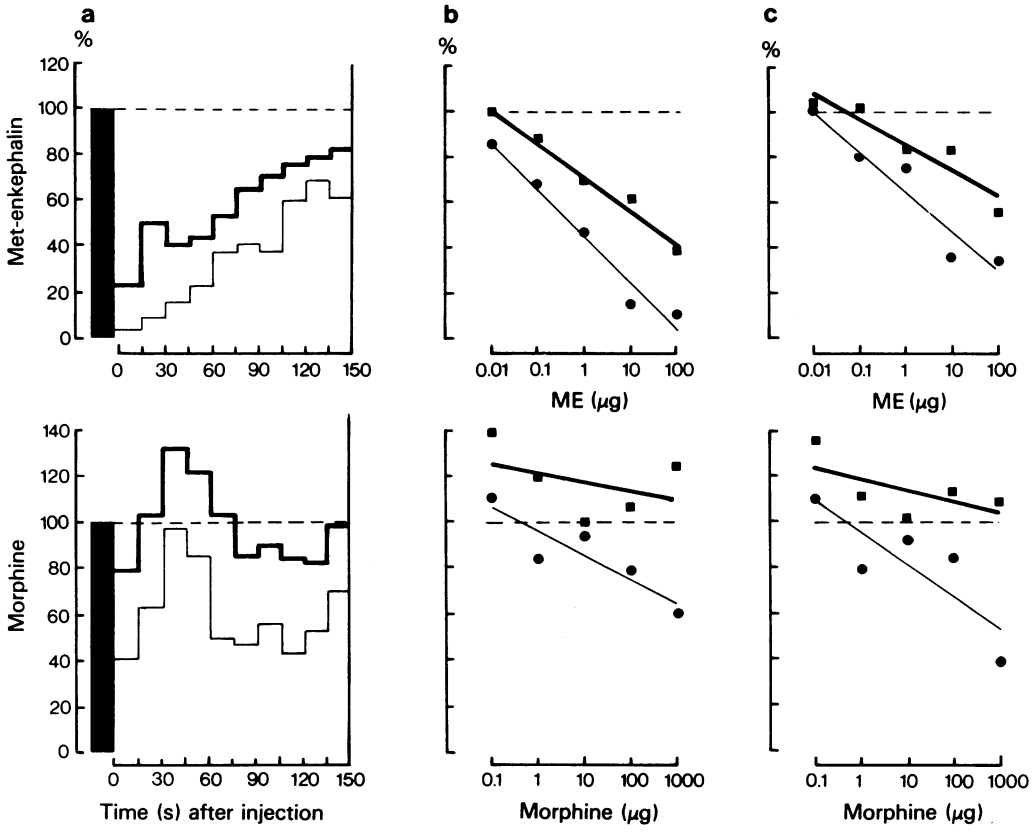

Figure 3 (a) Pooled data showing the effects of methionine-enkephalin (Met-enkephalin) 100 μg (n = 3) and morphine 1000 μg (n = 2) on spontaneous chemoreceptor discharge before (—) and after (—) injecting naloxone (0.2 mg i.c.). Black rectangles represent the control discharge (100%), values for Met-enkephalin being 3.7 ± 1.8 ct/s before and 3.1 ± 0.9 after naloxone, the corresponding values for morphine being 3.7 ± 1.7 and 4.9 ± 2.4 ct/s. See Figure 1 for further details. (b) is a plot of the total discharge over the 60 s post-injection period, expressed as a percentage of the total discharge which would have occurred in the same period if control discharge had continued unaltered (100% = dotted line), against $\log_{10}$ dose of methionine-enkephalin (ME) (pooled data from three experiments) or morphine (data from a single experiment) before (—) and after (—) naloxone (0.2 mg i.c.). Lines were fitted by the method of least squares. (c) is similar to (b), but the plot is of total discharge in the 150 s post-injection period and this includes the delayed inhibition seen with morphine.

Since spontaneous discharge was suppressed it was not possible, under these conditions, to investigate the effect of Met-enkephalin on the inhibitory response evoked by dopamine.

Responses to ACh, NaCN, CO_2 and dopamine were not appreciably affected by naloxone (0.2 mg i.c.).

Discussion

Our results indicate that enkephalin inhibited spontaneous chemoreceptor discharge by acting on naloxone-sensitive receptors in the carotid body. Morphine also acted at these receptors, but it was less potent than Met-enkephalin and caused a more variable inhibition which tended to be biphasic.

Although Met-enkephalin and morphine were able to inhibit spontaneous chemoreceptor discharge, they only slightly reduced excitatory responses evoked by ACh and NaCN, and potentiated dopamine-induced inhibition. These were long-term effects since the responses were not studied until 5 to 20 min after injections of Met-enkephalin or morphine. When responses were studied during an infusion of Met-enkephalin sufficient to reduce spontaneous chemoreceptor discharge substantially, the excitatory effect of NaCN was slightly reduced whereas responses to ACh and CO_2 were potentiated. These single-dose studies are difficult to interpret, particularly since

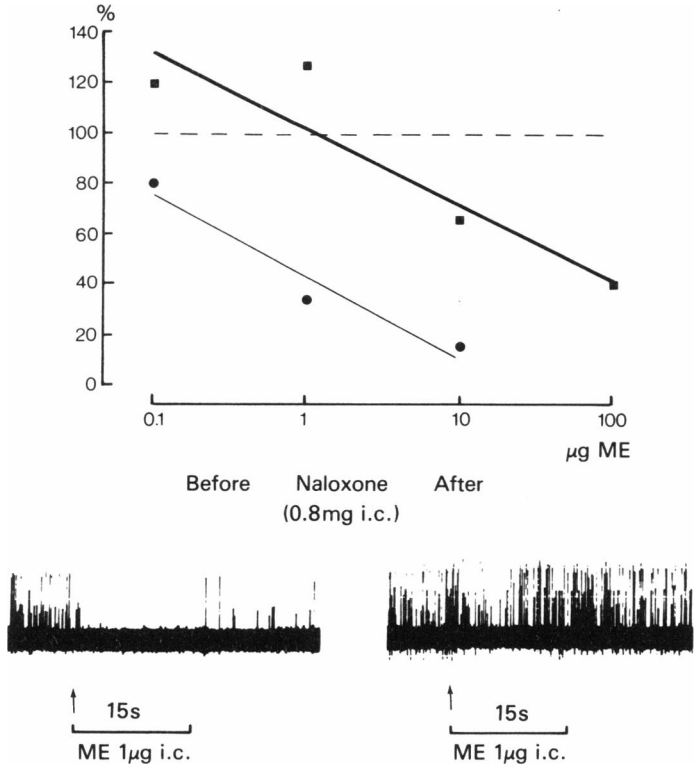

Figure 4 Effects of methionine-enkephalin (ME) on chemoreceptor discharge (plotted as a percentage of the total discharge in the 150 s post-injection period/control discharge × 150 s) before (—) and after (—) injecting naloxone (0.8 mg i.c.). Lines were fitted by the method of least squares. See Figure 3 for further details. Following this dose of naloxone, low doses of Met-enkephalin no longer inhibited chemoreceptor discharge; the tendency was for discharge to be increased. This can be clearly seen in the neurograms which show the effect of Met-enkephalin (1 μg i.c.), injected at the arrow, before and after naloxone (0.8 mg i.c.).

Table 1 Effects of methionine-enkephalin (Met-enkephalin) and morphine on evoked responses

Intracarotid injection of:	*ACh* (50 μg)	*CO_2-Locke* (0.3 ml)	*NaCN* (5 μg)	*Dopamine* (5 μg)	n
A Following morphine injections	83 ± 19	134 ± 11	89 ± 1	159 ± 61	3
B Following Met-enkephalin injections	77 ± 16	72 ± 17	63 ± 10	167 ± 24	5
C During Met-enkephalin infusions (50 μg/min i.c.)	137 ± 23	122 ± 9	84 ± 23	—	2

Pooled data from *n* experiments showing chemoreceptor responses ($\Delta\Sigma x$) (A) following injections of morphine and (B) following injections of Met-enkephalin. The results are expressed as mean percentages ± s.e. mean of the responses to the same doses administered before morphine or Met-enkephalin (i.e. pre-injection response = 100%). Injections were also made 60 s after starting a 65 s infusion of Met-enkephalin and responses evoked compared with pre-infusion values (C).

$\Delta\Sigma x = \Sigma x$ (total spike count during response period, t s) $- (\bar{x}.t)$ where $\bar{x}$ is the average pre-injection (control) discharge in ct/s.

Met-enkephalin may alter blood flow through the carotid body, but the important point is that whereas Met-enkephalin can reduce spontaneous chemoreceptor discharge, it has little effect on responses to intense stimuli of short duration. In the present experiments, the local concentration of Met-enkephalin/morphine may have been high enough to suppress the resting discharge but not that evoked by ACh, NaCN or CO_2.

Injection of the specific opiate antagonist, naloxone (see Sawynok, Pinsky & Labella 1979 for a review), slightly increased chemoreceptor discharge, an effect that was not simply due to reversal of residual Met-enkephalin or morphine chemo-depression because it was also observed when neither substance had been administered. This may mean that there is some tonic inhibition of chemoreceptor discharge by an opioid; alternatively, the effect could be secondary to changes (e.g. in BP) induced by naloxone acting elsewhere. The dose of naloxone used (0.2 mg) is adequate for reversing the fall in BP caused by morphine in cats (Feldberg & Wei, 1977; McQueen, unpublished observations) and for reversing the action of enkephalins in the cat substantia gelatinosa (Duggan, Hall & Headley, 1977). In the present experiments it reduced the chemoreceptor inhibition caused by Met-enkephalin and, to a greater extent, that caused by morphine. It is known that inhibition of neuronal firing caused by morphine is more readily antagonized by naloxone than is that caused by opioid peptides (North, 1979). Increasing the dose of naloxone to 0.8 mg caused an even greater reduction of the Met-enkephalin-induced inhibition of chemoreceptor discharge, and virtually abolished the morphine effect. Higher doses of naloxone could have been studied, but we were concerned that they might have exerted non-specific actions, or reversed the anaesthetic (Fürst, Foldes & Knoll, 1977; Arndt & Freye, 1979; Sawynok *et al.*, 1979). It seems reasonable to conclude that most of the chemoreceptor inhibition results from actions of Met-enkephalin and morphine on naloxone-sensitive receptors in the carotid body.

Low doses of morphine tended to increase spontaneous chemoreceptor discharge, an effect that was potentiated and also obtained with higher doses of morphine, as well as low doses of Met-enkephalin, after naloxone. Landgren, Liljestrand & Zotterman (1952) found that an intracarotid injection of 3 mg morphine hydrochloride in cats caused a moderate increase in small action potentials (probably chemoreceptors) recorded from the sinus nerve, and Eyzaguirre & Zapata (1968) showed that morphine caused a transient increase in discharge recorded from the *in vitro* carotid body preparation. Whether the excitation seen in the present study resulted from an action on naloxone-insensitive opiate receptors, or was caused by morphine/Met-enkephalin influencing substances in the carotid body, as occurs in other tissues (e.g. ACh (Paton, 1957), noradrenaline (Szerb, 1961; Snyder & Childers, 1979), 5-HT, or dopamine (Loh, Brase, Sampath-Khanna, Mar, Way & Li, 1976)), requires further investigation. So does the biphasic nature of the inhibitory response to morphine; could vascular changes be responsible for the transient return of discharge to control levels?

High doses of Met-enkephalin and morphine caused a fall in BP, and it is possible that some of the changes in chemoreceptor discharge might result from changes in blood flow through the carotid body. However, the fact that chemo-inhibitory actions of Met-enkephalin and morphine, (a) started within 1 to 2 s of the injection, (b) occurred following low doses which had no effect on BP and, (c) were greatly reduced by naloxone in doses which only slightly reduced the hypotensive effect, all argue against vascular effects contributing much to the inhibition, at least as far as the early part of the response is concerned. Further information could be obtained by studying the effect of Met-enkephalin on the *in vitro* carotid body preparation (Eyzaguirre & Lewin, 1961), which would eliminate the vascular complications.

The chemo-inhibitory effect of low doses of Met-enkephalin was very similar to that obtained with dopamine, (5 μg i.c.), although it should be noted that Met-enkephalin is 10 to 100 times more potent on a molar basis. However, despite the similarities, it is unlikely that Met-enkephalin acts directly, or, by releasing dopamine within the carotid body, indirectly at a dopamine receptor, because α-flupenthixol blocks the inhibitory action of exogenous dopamine (Docherty & McQueen, 1978) without affecting the inhibitory response to Met-enkephalin (McQueen, 1979).

Substance P is present in the cat carotid body (Cuello & McQueen, 1980) and causes an increase in chemoreceptor discharge on intracarotid injection (McQueen, 1980). It may be that Met-enkephalin is acting to inhibit the release of substance P within the carotid body in the same way as has been shown to occur in the CNS (Jessel & Iversen, 1977), the mechanism probably involving an action of Met-enkephalin on Ca^{2+} channels (Mudge, Leeman & Fischbach, 1979).

It has been suggested that adenosine might be the mediator of the neuro-inhibitory action of opiates (Sawynok & Jhamandas 1976; Stone & Perkins, 1979). Both adenosine (see Ribeiro, 1978) and morphine (Henderson, Hughes & Kosterlitz, 1975) decrease transmitter release in the central and peripheral nervous systems, and adenosine (Ribeiro, Sá-Almeida & Namorado, 1979), morphine (Guerrero-Munoz, Cerreta, Guerrero & Way, 1979), and β-endorphin (Guerrero-Munoz, Guerrero, Way & Li, 1979) decrease the uptake of calcium by synapto-

somes; Ca^{2+} is known to be involved in transmitter release (e.g. Katz & Miledi, 1968). Opioids might depress chemoreceptor activity by inhibiting the entry of Ca^{2+} needed for the release of putative sensory excitatory transmitter(s). Whether adenosine is the mediator of the Met-enkephalin or morphine-induced inhibition, and whether Met-enkephalin interacts with other substances in the carotid body (e.g. substance P, noradrenaline, 5-hydroxytryptamine, dopamine, ACh) requires investigation. Preliminary results (Cuello & McQueen, unpublished observations) suggest that enkephalin-like material is present in the carotid body.

In conclusion, the present results provide pharmacological evidence for the presence of an opiate receptor, or receptors, in the cat carotid body. What type of receptor this is (e.g. Lord, Waterfield, Hughes & Kosterlitz, 1977), where in the carotid body it is located, what the endogenous ligand is and where it originates, and the circumstances under which it is released, all need to be investigated before one can determine whether Met-enkephalin or other opioid peptides have a role as neurotransmitters or neuromodulators (Kosterlitz & Hughes, 1975; Snyder & Childers, 1979) in the cat carotid body chemoreceptors.

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