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Characterization of opioid receptors in the cat carotid body involved in chemosensory depression *in vivo*

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1 The effects of selective opioid receptor agonists and antagonists on neural discharge recorded from carotid body arterial chemoreceptors *in vivo* were studied in anaesthetized cats.

2 Mean ID_{50} values were determined for each agonist and used to assess chemodepressant potency on intracarotid (i.c.) injection in animals artificially ventilated with air. [Met]enkephalin, [Leu]enkephalin, [D-Ala^2, D-Leu^5]enkephalin and [D-Pen^2, D-Pen^5]enkephalin were more potent chemodepressants than [D-Ala^2, Me-Phe^4, Gly-ol^5]enkephalin, dynorphin (1–8) or ethylketocyclazocine; morphiceptin (μ-agonist) was inactive. The rank order of potency was compatible with the involvement of δ-opioid receptors in opioid-induced depression of chemosensory discharge.

3 ICI 154129, a δ-opioid receptor antagonist, was used in fairly high doses and caused reversible dose-related antagonism of chemodepression induced by [Met]enkephalin. It also antagonized depression caused by single doses of [Leu]enkephalin, [D-Ala^2, D-Leu^5]enkephalin, [D-Ala^2, Me-Phe^4, Gly-ol^5]enkephalin or dynorphin (1–8). ICI 174864, a more potent and selective δ-opioid receptor antagonist, also antagonized chemodepression induced by [Met]enkephalin or by the selective δ-receptor agonist [D-Pen^2, D-Pen^5]enkephalin.

4 Comparison of background or 'spontaneous' chemosensory discharge during the 30 min periods immediately before and after injecting ICI 174864 (0.1–0.2 mg kg^{-1} i.c.) showed a significant increase in discharge in one experiment, but in four others discharge was either unaffected or decreased after the antagonist, which argues against a toxic depression of chemosensors by endogenous opioids under resting conditions in our preparation.

5 Sensitivity of the carotid chemoreceptors to hypoxia (ventilating with 10% O_2) was increased significantly after ICI 174864, which could be taken as evidence that endogenous opioids depress chemosensitivity during hypoxia. In contrast, responsiveness to hypercapnia was reduced after the antagonist, implying that endogenous opioids may potentiate chemoreceptor sensitivity during hypercapnia.

6 The results obtained using 'selective' agonists and antagonists provide evidence that depression of chemosensory discharge caused by injected opioids involves a δ type of opioid receptor within the cat carotid body. Endogenous opioids may modulate arterial chemoreceptor sensitivity to physiological stimuli such as hypoxia and hypercapnia.

Introduction

The cat carotid body contains both [Met] and [Leu]enkephalin-like material (Lundberg *et al.*, 1979; Wharton *et al.*, 1980; Hansen *et al.*, 1982), and it has been shown that intracarotid injections or infusions of either [Met] or [Leu]enkephalin depress chemosensory discharge recorded from the cat carotid nerve *in vivo*, an effect that can be antagonized by relatively high doses of the opioid receptor antagonist naloxone (McQueen & Ribeiro, 1980; 1981a; McQueen, 1981; Pokorski & Lahiri, 1981). It appears, therefore, that the cat carotid body contains opioid peptides and possesses functional opioid receptors. This raises questions concerning the role of opioids in the process of arterial chemoreception and the characteristics of the opioid receptors involved in depression of chemosensory discharge.

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There seem to be at least three types of opioid receptor, namely μ, δ and κ (see Paterson *et al.*, 1983), so which of these is involved in opioid-induced chemosensory depression? It is known that morphine, preferentially a μ-receptor agonist, is not a very potent chemodepressant (McQueen & Ribeiro, 1980), and low doses of naloxone, which have a greater affinity for μ-receptors than for the other types of opioid receptor (Paterson *et al.*, 1983), are not very effective at antagonizing the chemodepressant action of [Met] or [Leu]enkephalin. This evidence suggests that μ-receptors are not directly involved in chemodepression induced by opioids and, because the enkephalins have greater affinity for δ- than for κ-sites, points to δ-receptors being responsible for the effects observed. However, the agonists [Met] and [Leu]enkephalin are not sufficiently selective to be used for receptor classification, and high doses of the antagonist naloxone will affect both δ- and κ-receptors, as well as μ-receptors (Magnan *et al.*, 1982). The available evidence is thus inadequate for classifying these opioid receptors in the carotid body.

New ligands have recently become available which, mainly on the basis of binding studies and experiments *in vitro*, are considered to be selective for the different types of opioid receptor (e.g. see Corbett *et al.*, 1984). We have used several of these agonists and antagonists in an attempt to characterize the opioid receptors in the cat carotid body that are involved in chemodepression *in vivo*. Some of the preliminary results have been presented previously (Kirby & McQueen, 1985; McQueen & Kirby, 1986).

Methods

The experiments were performed on cats anaesthetized with pentobarbitone (42 mg kg^{-1} i.p.), supplemented as required during the experiments, artificially ventilated with room air and paralysed with gallamine (3 mg kg^{-1} i.v.). Full details of the experimental procedures have been given previously (McQueen, 1977) and only a brief description follows. The lingual artery ipsilateral to the carotid nerve from which recordings were obtained was cannulated and the catheter tip positioned in the common carotid artery caudal to the bifurcation. Blood pressure was recorded from a cannulated femoral artery.

Electrical activity of chemoreceptor nerve fibres was recorded from filaments dissected from the peripheral end of a sectioned carotid (sinus) nerve, stored on FM tape, passed through a pulse height (window) voltage discriminator and quantified with the aid of a microcomputer. The ganglioglomerular (sympathetic) nerves were cut.

Drugs were dissolved in either modified Locke solution (McQueen, 1977) or 0.9% w/v aqueous sodium chloride. Drug injections were made in a volume of 0.1 ml into the lingual catheter (i.c.) and washed in with 0.2 ml Locke solution which had been equilibrated with 5% CO_2 : 95% air in a water bath at 37°C. Injections were completed within 2 s. The drugs used were: sodium pentobarbitone, gallamine triethiodide (May & Baker); [Met]enkephalin, [Leu]enkephalin, [D-Ala^2, D-Leu^5]enkephalin (DADLE), [D-Ala^2, Me-Phe^4, Glyol^5]enkephalin, (DAGOL), dynorphin-(1–8)-octapeptide (CRB); ethylketocyclazocine (kindly donated by Sterling-Winthrop), [D-Pen^2, D-Pen^5]enkephalin (Peninsula Laboratories); (±)-naloxone hydrochloride, morphiceptin hydrochloride (Sigma); ICI 154129 (N,N-diallyl-Tyr-Gly-Gly-ψ-(CH_2)S-Phe-Leu-OH, arginine salt) and ICI 174864, (N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH, arginine salt) kindly donated by ICI, Macclesfield.

Results

Agonists

[Met]enkephalin caused dose-related depression of chemosensory discharge when injected intra-arterially close to the carotid body *in vivo* (e.g. Figures 1–3), in confirmation of results from previous studies with opioids (see Introduction). The ID_{50} (dose causing a 50% reduction in discharge during the 30 s period immediately following injection) was calculated from data, such as those shown in Figures 2 and 3, for opioid agonists and the values obtained are given in Table 1.

The mean ID_{50} for [D-Pen^2, D-Pen^5]enkephalin was significantly higher than that of [Met]enkephalin ($P<0.05$, Wilcoxon two-sample test); the mean ID_{50} values for [D-Ala^2, D-Leu^5]enkephalin and [Leu]enkephalin were also higher, but because they were associated with larger standard errors, they did not differ significantly from that of [Met]enkephalin ($P>0.05$). The ID_{50} values for DAGOL, dynorphin (1–8) and ethylketocyclazocine were all significantly higher than that of [Met]enkephalin ($P<0.01$). There was no evidence for any marked chemoexcitation on injecting δ-, μ- or κ-receptor agonists, but in some experiments an initial low dose of a δ-receptor agonist caused a slight increase in discharge. Higher doses (>25–100 μg) tended to desensitize the preparation transiently, and were therefore avoided as far as possible. In several experiments high doses of [Met]enkephalin were followed by loss of sensitivity to opioids which lasted for as long as the experiment continued (i.e. 3–4 h), and in these cases the chemoreceptors also became very insensitive to injections of sodium cyanide (chemoexcitant) or dopamine (chemodepressant), although they did respond very sluggishly to lowering the Pa_{O_2} by ventilating the animal with 10% O_2 : 90%

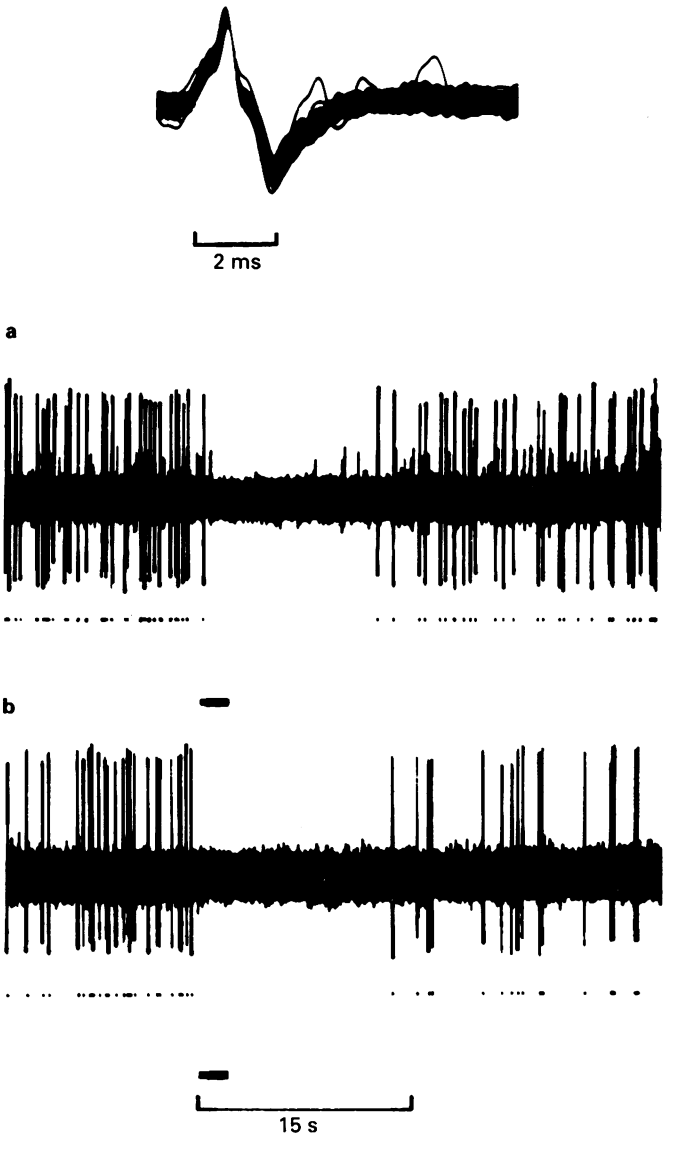


Figure 1 Neurograms illustrating the effects of the δ-opioid receptor agonist [D-Pen^2, D-Pen^5]enkephalin 1 μg i.c. (a) and [Met]enkephalin 0.1 μg i.c. (b) on chemosensory discharge recorded from an anaesthetized cat artificially ventilated with air. The upper panel shows 50 superimposed sweeps of the large action potential, and the output from a voltage discriminator set to count only this single unit is shown as a row of dots below the neurogram. Injections are represented by the horizontal bars.

N_2. This long-lasting effect appeared to be correlated with damage to the carotid body vasculature.

Antagonists

ICI 154129 Responses to [Met]enkephalin were obtained before and after injecting single doses of ICI 154129 (0.1–10 mg kg^{-1}), as illustrated in Figure 3, and ID_{50} values were calculated from dose-response lines. The antagonist was not very potent, but at doses of 1 mg kg^{-1} and above it was able to cause a dose-related reduction in the chemodepressant action of [Met]enkephalin, and this effect lasted for 1–2 h. Cumulative doses of 0.1, 1 and 10 mg kg^{-1} ICI 154129

Table 1 Rank order of potency of opioid agonists as chemodepressants, based on mean ID_{50} values (± s.e.mean), in cats artificially ventilated with air

Agonist	*Dose range* (μg i.c.)	*ID_{50}* (nmol)	n
[Met]enkephalin	0.001–100	0.67 ± 0.13	16
[D-Pen^2, D-Pen^5] enkephalin	0.001–100	1.58 ± 0.40	5
[D-Ala^2, D-Leu^5] enkephalin	0.001–100	2.16 ± 1.30	3
[Leu]enkephalin	0.001–100	2.60 ± 1.45	5
[D-Ala^2, Me-Phe^4, Gly-ol^5]enkephalin	0.001–100	17.5 ± 4.7	5
Dynorphin (1–8)	0.01 –100	48.2 ± 15.9	5
Ethylketocyclazocine	0.01 –100	2399 ± 1353	5
Morphiceptin	0.01 –100	–†	3

†No dose-related chemodepression, so an ID_{50} value could not be obtained. n = number of experiments.

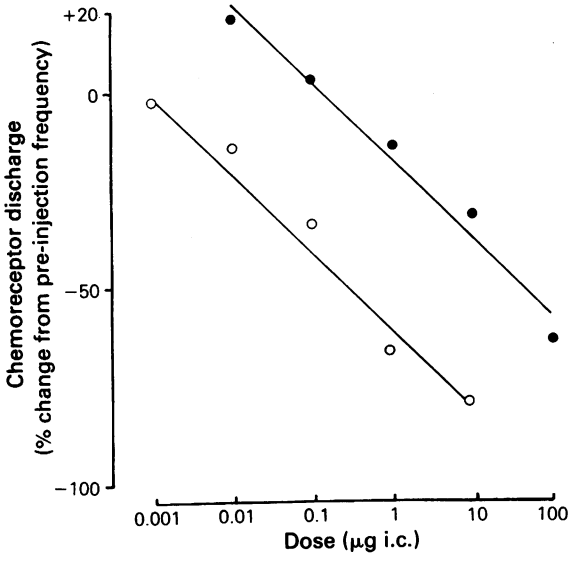


Figure 2 Dose-response lines, fitted to data by the method of least squares, showing the dose-related depression of chemosensory discharge caused by intra-carotid (i.c.) injection of [Met]enkephalin (○) or dynorphin (1–8) (●) during the same experiment in an anaesthetized cat artificially ventilated with air. Chemoreceptor discharge was averaged over the 30 s post-injection period and expressed as the percentage change from the pre-injection discharge frequency. ID_{50} values were calculated from the lines.

were used initially, but in later experiments the lower doses were eliminated; in some experiments the preparation deteriorated before the high dose of antagonist could be given. The mean ID_{50} for [Met]enkephalin-induced chemosensory depression was 0.89 ± 0.16 (± s.e.mean, n = 9) nmol before the antagonist, and after 0.1, 1 and 10 mg kg^{-1} of ICI 154129 the corresponding values were 1.01 ± 0.38 (n = 3), 4.32 ± 1.2 (n = 4) and 73 ± 26 (n = 6) nmol, respectively. The 10 mg kg^{-1} dose also antagonized chemodepression evoked by single doses of [Leu]enkephalin, DADLE, DAGOL and dynorphin (1–8); ID_{50} values were not obtained for these substances because of practical problems associated with deriving full dose-response curves for more than one agonist before and after administering the antagonist. High doses of μ- or κ-receptor agonists caused slight chemoexcitation after ICI 154129 in some, but not all, experiments.

In order to assess the effect of ICI 154129 itself on chemosensory discharge, measurements were made during the 30 s period immediately following injection of 10 mg kg^{-1} of the antagonist, and discharge expressed as a percentage change from the pre-injection frequency. After 1 mg kg^{-1} i.c. discharge fell by 11 ± 4%, and after an additional 10 mg kg^{-1} it fell by 39 ± 24% (n = 6). The longer-term effects of the antagonist on chemosensory discharge were evaluated by averaging the pre-injection frequency from tests performed in the 30 min period preceding administration of the 10 mg kg^{-1} dose, and comparing the values with those obtained in the same period post-antagonist. The results are shown in Figure 4.

ICI 174864 A series of experiments was performed using the more potent δ-opioid receptor antagonist ICI 174864. This analogue antagonized the chemodepressant effects of both [Met]enkephalin (e.g. Figure 3) and the selective δ-opioid antagonist [D-Pen^2, D-Pen^5]enkephalin when used in doses of 0.1–0.5 mg kg^{-1} i.c. The mean ID_{50} for [Met]enkephalin was 0.51 ± 0.17 nmol (n = 5) before the

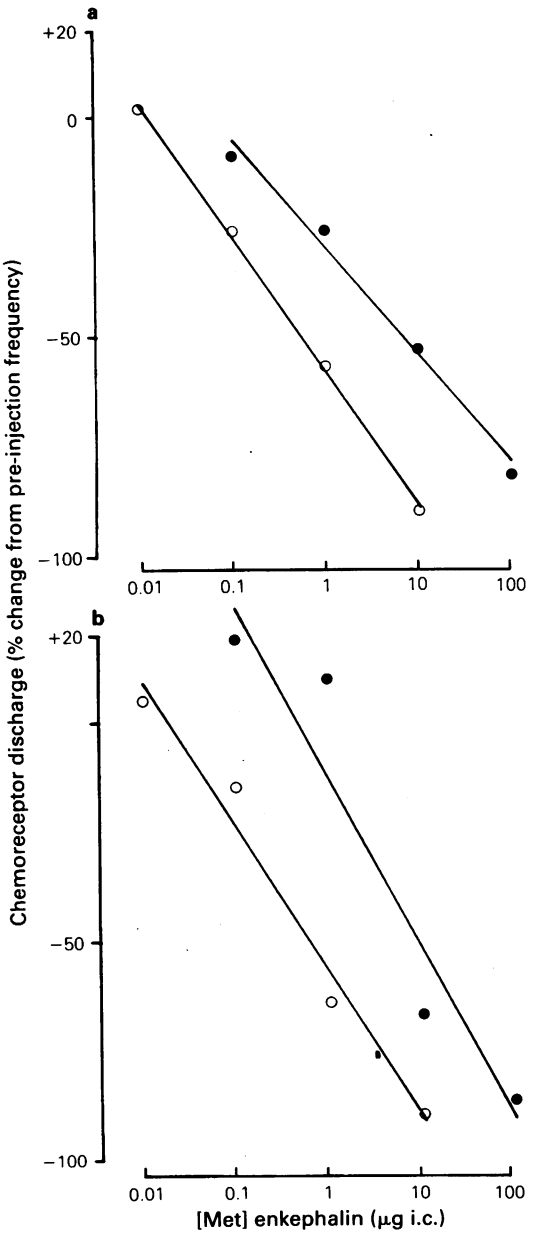


Figure 3 Dose-response lines fitted by the method of least squares to data from two experiments showing dose-related chemodepression caused by i.c. injection of [Met]enkephalin before (○) and after (●) either ICI 154129, 10 mg kg^{-1} i.c. (a) or ICI 174864, 0.2 mg kg^{-1} i.c. (b). In both cases the antagonist shifted the dose-response line to the right.

antagonist, 4.05 ± 1.5 nmol ($n = 4$) after 0.1 mg kg^{-1} i.c., and 150 ± 81 nmol ($n = 5$) after 0.2 mg kg^{-1}. In two experiments with [D-Pen^2, D-Pen^5]enkephalin the mean ID_{50} was 1.65 nmol before ICI 174864, and 11 nmol after 0.1 mg kg^{-1} of the antagonist. ICI 174864 was 10–20 times more potent than ICI 154129 in antagonizing chemodepression induced by [Met]enkephalin, and the effect lasted for 60–90 min.

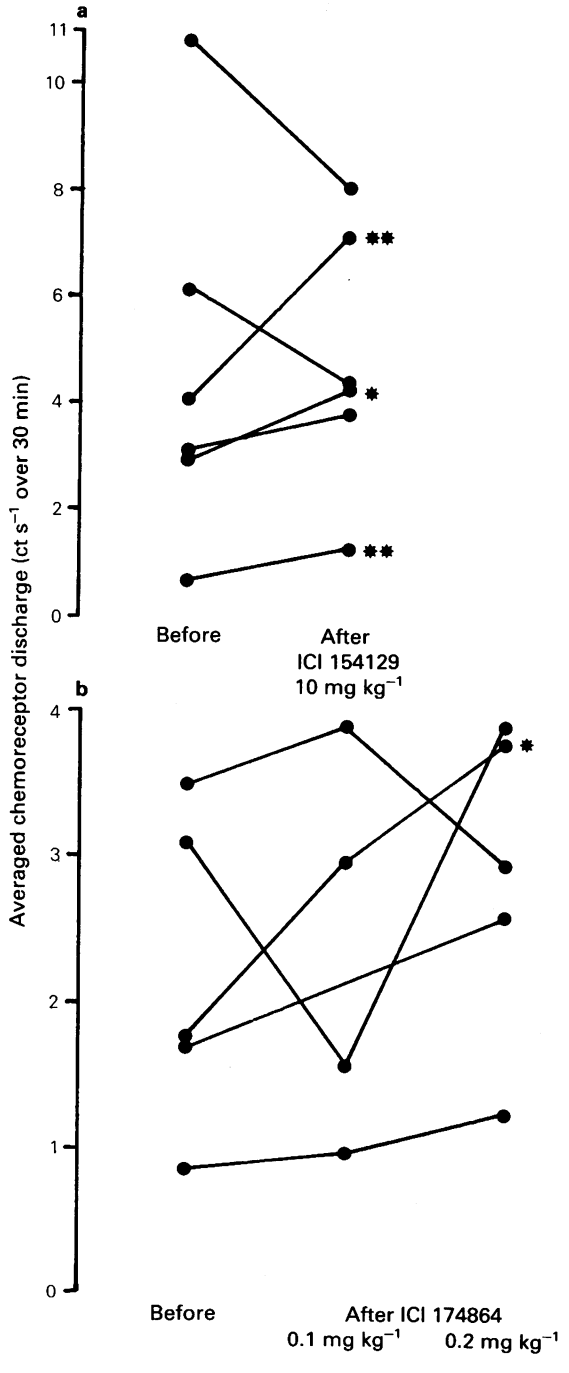
ICI 174864 itself had little effect on chemosensory discharge; during the 30 s period immediately following injection of the antagonist discharge increased by 0.5 ± 23% (0.1 mg kg^{-1}, $n = 4$) and decreased by 23 ± 16% (0.2 mg kg^{-1}, $n = 5$), but neither of these values differed significantly from the effect associated with injecting the drug vehicle. The longer-term effects of the antagonist were assessed as described above for ICI 154129, and the data are shown in Figure 4. Neither ICI 174864, nor ICI 154129, had any significant effect on arterial blood pressure.

Physiological stimuli

Hypoxia Ventilating the animal with 10% O_2 : 90% N_2 instead of air for 4 min provided a strong submaximal stimulus to the carotid body chemoreceptors. The rate at which chemosensory discharge increased (dynamic component) and the steady-state (static component) when the response to hypoxia had plateaued (usually about 3 min after switching to hypoxic gas) were determined in four cats before and after injecting ICI 174864 (0.1–0.2 mg kg^{-1} i.c.). The results obtained are shown in Figure 5 where data have been normalised with respect to the plateau or steady-state frequency measured during hypoxia in the pre-antagonist state (= 100%), to allow for differences between preparations in basal discharge frequency and in sensitivity to hypoxia.

The pooled data indicated that discharge increases more rapidly in response to the hypoxic stimulus following administration of ICI 174864, and the steady-state is achieved sooner. However, there was some variability, and consideration of individual experiments showed that in three experiments (Figure 5a) discharge frequency post-antagonist was significantly greater than that observed during hypoxia in the control or pre-antagonist state ($P < 0.01$ in each case; Wilcoxon signed ranks test performed on paired values, pre- vs post-antagonist, of averaged discharge in the 12 successive 15 s intervals during the first 180 s of the hypoxic stimulus). In the fourth experiment (Figure 5b) the opposite was the case, with discharge post-antagonist being significantly lower ($P < 0.05$); however, basal discharge was also considerably lower after ICI 174864 in this experiment.

There was no significant difference in measurements of arterial blood gas tensions made before and after ICI 174864 during air-breathing or 180 s after switch-



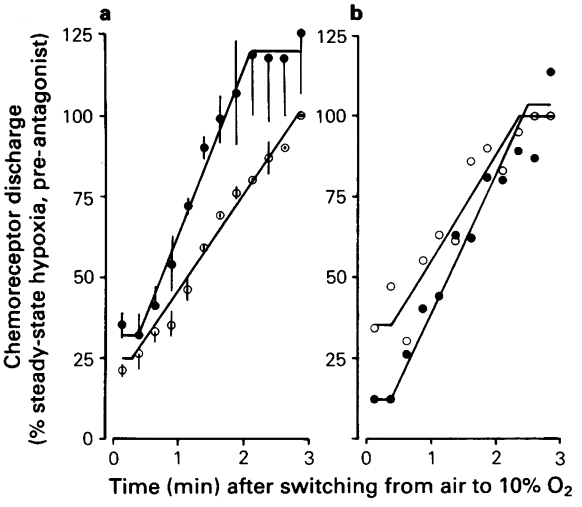


Figure 5 Chemosensory discharge, expressed as a percentage of steady-state discharge (=100%) during artificial ventilation of anaesthetized cats with 10% O_2 : 90% N_2 in the pre-antagonist or control state, plotted against time after switching to the hypoxic gas mixture. Results from three animals were similar, and the pooled data are shown in (a), whereas the other experiment, shown in (b), differed in that values after the δ-opioid antagonist ICI 174864, 0.2 mg kg^{-1} i.c. (●) were lower than in the control state (○), which is the opposite to that observed in (a). In all four experiments the slope of the lines was increased after the antagonist. In (a) vertical lines indicate s.e.mean.

ing to 10% O_2. Mean values for blood samples (± s.e.mean; kPa for gases, $n = 4$) during air breathing before and after (value in parentheses) the antagonist were: Pa_{O_2} 12.63 ± 0.40 (12.74 ± 0.80), Pa_{CO_2} 3.75 ± 0.21 (3.74 ± 0.29) and pH 7.29 ± 0.01 (7.30 ± 0.02). During hypoxia the corresponding values were: Pa_{O_2} 5.69 ± 0.53 (5.56 ± 0.52), Pa_{CO_2} 3.76 ± 0.16 (3.95 ± 0.05) and pH 7.32 ± 0.02 (7.34 ± 0.03).

Figure 4 Chemosensory discharge during air-breathing was averaged for the 30 min periods before and after injecting either ICI 154129 (a) or ICI 174864 (b). Mean discharge recorded in individual animals pre- and post-antagonist are joined by lines. (*$P < 0.05$; **$P < 0.01$; Wilcoxon two sample test; pre- vs post-antagonist). Overall, discharge averaged 4.6 ± 1.4 ct s^{-1} before ICI 154129 and 4.8 ± 1.0 ct s^{-1} post-antagonist. The corresponding values for ICI 174864 were 2.2 ± 0.3 ct s^{-1} (before), 2.4 ± 0.3 ct s^{-1} (after 0.1 mg kg^{-1} i.c.) and 2.9 ± 0.3 ct s^{-1} (after 0.2 mg kg^{-1} i.c.)

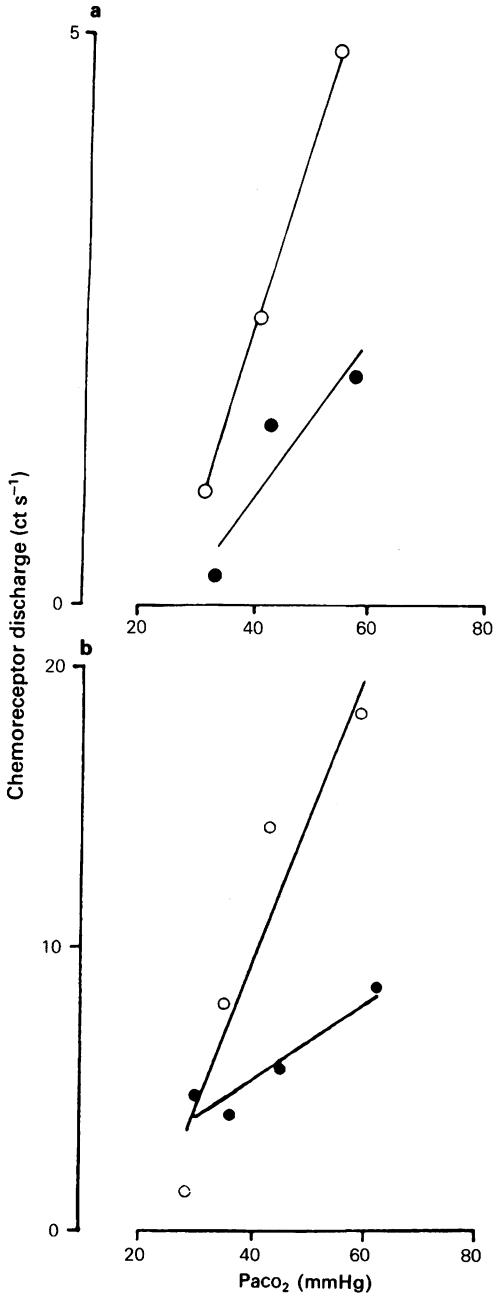


Figure 6 Steady-state chemoreceptor discharge, averaged over 30 s, at different levels of $PaCO_2$ achieved by adding CO_2 to the inspired gas mixture in artificially ventilated anaesthetized cats. (a) and (b) show results obtained from two experiments, and in both cases discharge measured before (○) tended to be higher than that observed after (●) a single dose of the δ-receptor antagonist ICI 174864 (0.2 mg kg^{-1} i.c.).

In all four experiments the slope of the line relating discharge frequency to duration of the hypoxic stimulus was greater after ICI 174864. Values were 0.51 ± 0.03 ($n = 4$) % steady-state s^{-1} before and 0.82 ± 0.17 ($n = 4$) after the antagonist ($P < 0.05$, Wilcoxon two-sample test). Thus, chemosensitivity to hypoxia was enhanced after administering the δ-opioid receptor antagonist.

Hypercapnia The $PaCO_2$ was altered in two experiments by adding CO_2 to the inspired gas mixture (20% O_2 : 80% N_2) and adjusting the oxygen content to maintain a constant PaO_2. Chemosensory discharge was averaged over a 30 s period during steady-state conditions at each of the $PaCO_2$ levels, and the sequence was repeated after injecting ICI 174864 (0.2 mg kg^{-1} i.c., single dose).

The results obtained are shown in Figure 6 from which it can be seen that in both experiments the slope of the line relating chemosensory discharge to $PaCO_2$ was decreased after the antagonist, which suggests that chemosensitivity to hypercapnia was reduced by the δ-receptor antagonist.

Discussion

The present results confirm previous work showing that [Met] and [Leu]enkephalin, which both appear to be present in the cat carotid body, cause a dose-related depression of chemosensory discharge when injected i.c. close to the cat carotid body *in vivo* (McQueen & Ribeiro, 1980; 1981a; McQueen, 1981; Pokorski & Lahiri, 1981). The suggestion that opioid-induced chemosensory depression may be mediated via δ-opioid receptors (McQueen, 1983; 1985) is supported, within the limits associated with studying receptors *in vivo*, by the evidence from our study using 'selective' opioid receptor agonists and antagonists, as discussed below.

Opioid agonists

Various agonists which are regarded as selective, within certain limits, for different types of opioid receptor now exist (e.g. Barnard & Demoliou-Mason, 1983; Paterson *et al.*, 1983). We studied the chemodepressant action of some of these compounds and expressed their potency on i.c. injection in terms of ID_{50} values derived from dose-response lines. [Met]enkephalin was used as the 'standard' against which responses to analogues were compared because the cat carotid body contains three times more [Met] than [Leu]enkephalin-like material (Wharton *et al.*, 1980). The rank order of potency as chemodepressants showed that analogues with high affinity for δ-opioid receptors were more active than those which were

predominantly μ- or κ-receptor agonists; the μ-receptor agonist morphiceptin (Chang *et al.*, 1981) was inactive in the doses used. However, opioids such as [Met]enkephalin, [Leu]enkephalin, DADLE or DAGOL can affect more than one site (e.g. Gillan & Kosterlitz, 1982; Paterson *et al.*, 1983; Corbett *et al.*, 1984) which makes it difficult to interpret results obtained using them *in vivo*.

During the course of our investigation we were able to obtain some [D-Pen^2, D-Pen^5]enkephalin, which is a selective δ-opioid receptor agonist (Mosberg *et al.*, 1983; Corbett *et al.*, 1984; Cowan *et al.*, 1985) exhibiting highly selective binding to δ-sites in homogenates of rat or guinea-pig brain (Cotton *et al.*, 1985). [D-Pen^2, D-Pen^5]enkephalin caused dose-related depression of chemosensory discharge in our experiments, although it was shorter-acting and less potent than [Met]enkephalin. Overall, the results obtained using agonists are compatible with δ-opioid receptors being involved in chemosensory depression. There was no evidence for any significant chemoexcitation on injection of δ-, μ- or κ-receptor agonists.

Opioid antagonists

We studied the effects of selective δ-opioid receptor antagonists on opioid-induced chemodepression in order to obtain further evidence regarding the nature of the opioid receptor involved. Initially we used ICI 154129, which is a selective δ-receptor antagonist (Shaw *et al.*, 1982). This opioid analogue was not very potent, but was capable of causing dose-related antagonism of chemosensory depression elicited by [Met]enkephalin. It also antagonized chemodepression caused by [Leu]enkephalin, DADLE, DAGOL and dynorphin (1–8), which suggests that these agonists were reducing chemosensory discharge via actions at δ-receptors. This suggestion is tentative because it is based on evidence from experiments in which only single doses of the analogues were administered before and after ICI 154129.

ICI 174864 is a more potent and selective δ-opioid receptor antagonist than ICI 154129 (Corbett *et al.*, 1984; Cotton *et al.*, 1984; 1985; Cowan *et al.*, 1985). It was qualitatively similar to ICI 154129, but 10–20 times more potent, in antagonizing opioid-induced chemodepression; the analogue inhibited the effects of [Met]enkephalin and [D-Pen^2, D-Pen^5]enkephalin on chemosensory discharge, and this action was dose-related. Thus, the results obtained using these selective δ-opioid antagonists reinforce the evidence that δ-opioid receptors are involved in opioid-induced depression of chemosensory discharge.

Experiments using the cat carotid body *in vitro* preparation (Monti-Bloch & Eyzaguirre, 1985) showed that [Met]enkephalin induced variable effects on chemosensory discharge following bolus application; low doses caused excitation, whereas higher doses caused depression. Superfusion with [Met]enkephalin depressed sensory discharge; and this effect was antagonized by naloxone. There is no information concerning the effects of naloxone on the excitation or inhibition elicited by bolus application of the opioid *in vitro*. If opioids have excitatory actions on the carotid chemoreceptors, these should be unmasked by antagonizing their chemodepressant effect – unless excitation is mediated via the same type(s) of opioid receptor. There was no tendency for opioids to cause marked chemoexcitation following administration of either ICI 154129 or ICI 174864 in our study. In some experiments a slight variable dose-related increase in discharge was obtained with ethylketocyclazocine and dynorphin (1–8), much the same as that observed with β-endorphin following naloxone (McQueen & Ribeiro, 1981b). It is not clear whether these very variable excitatory effects result from actions mediated via opioid receptors, or are non-specific actions of opioids administered in high doses. The fact that we were unable to detect any appreciable chemoexcitation with opioids *in vivo* may be attributable to factors operating in our preparation (e.g. presence of anaesthetic agent, neuromuscular blocking drug, circulating agents – including opioids and catecholamines) which might affect receptors involved in excitation. Alternatively, the *in vitro* preparation may be sensitive to this action of opioids because of differences from the situation *in vivo*, such as in the time-course and intensity of stimulus, the types of receptors directly or indirectly affected, the inactivation of peptides, desensitization of receptors, or the levels of endogenous substances being released from cells in the carotid body.

[Met]enkephalin-like material is present in glomus (type 1) cells (Lundberg *et al.*, 1979; Wharton *et al.*, 1980) where it may be stored together with catecholamines (Hansen *et al.*, 1982). Opioid receptors could be associated with one or more elements of the sensory receptor complex (e.g. glomus cells, sustentacular cells, afferent nerves, efferent nerves, blood vessels). Direct effects on the vasculature are unlikely to account for chemodepression elicited by opioids because the effect is almost instantaneous (e.g. Figure 1) and is also obtained *in vitro*. It has been found that [Met]enkephalin depolarizes the glomus cell membrane (cited by Monti-Bloch & Eyzaguirre, 1985), which may mean that at least some opioid receptors are situated on glomus cells. It should be possible to localize opioid receptors within the carotid body by studying the binding of selective radioligands using autoradiography, and this would indicate whether particular types of opioid receptor are present on some or all glomus cells and associated nerve terminals.

How opioids depress chemosensory discharge also remains to be established. In other systems these

peptides have been shown to inhibit neuronal firing (see North, 1979) and to depress the release of neurotransmitters (e.g. Yaksh *et al.*, 1980), actions which may result from effects of opioids on, for example, Ca^{2+} uptake (Guerrero-Munoz *et al.*, 1979), increased K^+ conductance (via μ-receptors (Williams *et al.*, 1982) or δ-receptors (North, 1986)) and/or changes in activity of adenylate cyclase (West & Miller, 1983). To explain chemodepression it would be simplest to assume that release of a chemo-excitant is inhibited in the carotid body by a process involving δ-opioid receptors (e.g. Mulder *et al.*, 1984). A more complex possibility is that opioids disinhibit a process that normally attenuates release of an inhibitory transmitter (e.g. Nicoll *et al.*, 1980). Obviously further experiments will be needed to study how opioids affect chemosensory discharge, and these could be based on knowledge gained from other systems (e.g. nicotinic stimulation activates release and biosynthesis of [Met]enkephalin in adrenal chromaffin cells – Eiden *et al.*, 1984; acetylcholine release is inhibited by δ-opioid receptor agonists in striatal slices – Mulder *et al.*, 1984).

Our results provide some evidence concerning the physiological role of opioids and opioid receptors in the cat carotid body. The finding that δ-opioid receptor antagonists had rather insignificant effects on chemosensory discharge in animals ventilated with air is in accord with results obtained using naloxone *in vivo* (McQueen & Ribeiro, 1980; Pokorski & Lahiri, 1981); naloxone (10^{-6}M) caused a 26% increase in chemosensory discharge *in vitro* (Monti-Bloch & Eyzaguirre, 1985). It appears, therefore, that there is little or no tonic inhibition of chemosensory discharge via δ-opioid receptors during normoxia in our preparation. Responsiveness to hypoxia was, however, increased after the δ-receptor antagonists had been given, and similar effects have been obtained with naloxone *in vivo* (Pokorski & Lahiri, 1981). This suggests that opioids are released during hypoxia and act at δ-receptors to attenuate chemosensory discharge. The opioids may be released within the carotid body, but could also reach it via the circulation (e.g. following release from the adrenals). In the case of hypercapnia, only two experiments were performed, but they both showed a reduction in sensitivity to hypercapnia after administration of the δ-receptor antagonist. This may mean that opioids normally potentiate the sensitivity of carotid chemoreceptors to CO_2, although naloxone had no appreciable effect on chemosensitivity to hypercapnia during hyperoxia *in vivo* (Pokorski & Lahiri, 1981). [Met]enkephalin superfusion reduced the chemoreceptor response to CO_2 in seven tests *in vitro*, increased it in two tests, and had no effect in another test (Monti-Bloch & Eyzaguirre, 1985). Further studies are needed to clarify the role of opioids in chemoreception, but it appears that they may modulate chemosensitivity by acting, at least in part, via δ-opioid receptors in the cat carotid body.

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