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Effects of opioid peptides and morphine on histamine-induced catecholamine secretion from cultured, bovine adrenal chromaffin cells

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1 The effect of opioid peptides and morphine on histamine-induced catecholamine secretion has been studied in monolayer cultures of dispersed, bovine adrenal chromaffin cells.

2 Histamine-induced a dose-dependent secretion of both adrenaline and noradrenaline with a threshold dose of approximately 5 nM, an EC_{50} of 150 nM and maximal secretion at 10 μM.

3 Catecholamine secretion induced by 1 μM histamine was completely dependent on extracellular calcium, was inhibited in a dose-dependent manner by mepyramine (1 nM–1 μM), and was unaffected by cimetidine (10 μM) and hexamethonium (0.1 mM).

4 Dynorphin-1-13 (1 nM–20 μM), metorphamide (0.1 nM–10 μM), morphine (1 nM–0.1 mM) and diprenorphine (1 nM–0.1 mM) each had no effect on adrenaline or noradrenaline secretion induced by 1 μM histamine.

5 The characteristics of histamine-induced catecholamine secretion from bovine adrenal chromaffin cells were similar to those reported previously for cat and rat adrenal medulla being calcium-dependent and mediated by H_1 histamine-receptors. The results with opioid peptides and morphine suggest that endogenous adrenal opioid peptides do not act on the opioid binding sites found on adrenal medullary chromaffin cells to modify their secretory response to histamine.

Introduction

In the last six years, more than 30 different opioid peptides have been identified in the mammalian adrenal medulla (Lewis & Stern, 1983; Udenfriend & Kilpatrick, 1983). Immunocytochemical studies have shown that these peptides are present both in chromaffin cells and in splanchnic nerve terminals in the adrenal medulla (Schultzberg *et al.*, 1978; Livett *et al.*, 1982). Opioid peptides are released from the adrenal into the circulation *in vivo* upon stimulation of the splanchnic nerve (Hexum *et al.*, 1980; Chaminade *et al.*, 1984) and can be released *in vitro* from isolated chromaffin cells by stimulation with nicotinic agonists or high potassium ion concentrations (Livett *et al.*, 1981; Rossier *et al.*, 1981). Adrenal medullary chromaffin cells have been shown to possess high-affinity, stereospecific opioid binding sites (Chavkin *et al.*, 1979; Kumakura *et al.*, 1980; Costa *et al.*, 1981; Lemaire *et al.*, 1981; Saiani & Guidotti 1982; Castanas *et al.*, 1985a,b) suggesting that opioid peptides released from splanchnic nerve terminals or from the adrenal medullary chromaffin cells may have a physiological role within the adrenal medulla.

In previous work, the ability of opioid peptides and opiate alkaloids to modify nicotinic secretory effects on isolated bovine adrenal chromaffin cells has been studied (Kumakura *et al.*, 1980; Costa *et al.*, 1981; 1983; Lemaire *et al.*, 1981; Dean *et al.*, 1982; Saiani & Guidotti 1982; Marley *et al.*, 1986a,b). It was found that opioid receptor agonists are capable of inhibiting nicotinic stimulation of catecholamine (CA) or ATP secretion from adrenal medullary cells. However, the peptides tested were all very weak in these actions and were poorly antagonized by classical opioid receptor antagonists, such as naloxone and diprenophine (Dean *et al.*, 1982; Marley *et al.*, 1986a,b). Furthermore, the actions of opiate alkaloids were also very weak and did not show stereoselectivity (Dean *et al.*, 1982). Consequently, it is unlikely that the classical opioid 'receptors' identified on bovine chromaffin cells by ligand-binding work act physiologically to modify nicotinic stimulation of CA release.

In neonatal animals, before adrenal innervation

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occurs, naloxone (5 mg kg^{-1}, s.c.) potentiates the non-neurogenic depletion of catecholamines induced by reserpine, while methadone (2.5 mg kg^{-1}, s.c.) inhibits this response (Chantry *et al.*, 1982). This suggests that opioid compounds may act directly on the adrenal medulla to modify its response to non-neurogenic stimuli. By contrast, in the mature rat, morphine and methadone evoke adrenal catecholamine secretion by a central reflex which results in a depletion of adrenal catecholamines (Yoshizaki, 1973; Slotkin *et al.*, 1980). Similar results have been obtained in adult dogs where morphine acts indirectly because it fails to cause secretion of catecholamines in animals with denervated adrenals (Costa *et al.*, 1981). To see whether the adrenal medullary receptors for opioid peptides have a functional role *in vivo* in the adult, Costa *et al.* (1981) carried out experiments in anaesthetized dogs in which the peripheral stump of the splanchnic nerve was stimulated electrically and blood samples taken from the cannulated adrenal vein were analyzed for catecholamines and opioid peptides (Hanbauer *et al.*, 1982). Splanchnic nerve stimulation produced a frequency-dependent secretion both of catecholamines and opioid peptides (Costa *et al.*, 1983; 1984). Injection of diprenorphine (a blocker of adrenal opioid responses), increased further the secretion of low molecular weight opioid peptides and of catecholamines. Hence, an inhibitory regulatory mechanism similar to that proposed from studies on isolated adrenal chromaffin cells *in vitro*, appears to operate *in vivo*. The possibility could not be excluded however that the release of catecholamines was inhibited by the secreted adrenal opioids acting on presynaptic receptors located in splanchnic nerve axons.

Adrenal CA secretion is, however, not solely under the control of nicotinic receptors on the chromaffin cells. A number of endogenous secretagogues other than acetylcholine are known to play a physiological role in regulating adrenal medullary secretion. It is possible that adrenal opioid peptides may act to modify the CA secretion produced by one of these other secretagogues. In the present study, we have tested the ability of opioid peptides and morphine to modify CA release evoked from cultured, bovine adrenal chromaffin cells by histamine.

Methods

Preparation of primary cultures of dispersed, bovine, adrenal chromaffin cells

Histamine-induced adrenal CA secretion was studied in primary monolayer cultures of bovine isolated adrenal chromaffin cells. Cells were prepared by collagenase digestion of adrenal glands, purified by Percoll (TM) density gradient centrifugation and cultured in 24 well plastic culture dishes pre-coated with rat tail collagen (see Livett 1984; Livett *et al.*, 1986b for full details). Cells were used for catecholamine release experiments after being cultured for 3 days, to allow attachment of the cells.

Stimulation of chromaffin cell catecholamine release

On the third day after plating, culture dishes were removed from the incubator and equilibrated to room temperature (20–22°C) for 5 min. Each well then received either three or four incubations in a medium consisting of (mM): NaCl 154, KCl 2.6, K_2HPO_4 2.15, KH_2PO_4 0.85, $MgSO_4$ 1.18, D-glucose 10, $CaCl_2$ 2.2, bovine serum albumin 0.5%. In experiments where the wells received three incubations, the first two incubations were 5 min washes, and the third a 20 min stimulation period with histamine. In the experiments assessing the calcium-dependence of histamine-induced CA release, all three incubations were performed in medium without $CaCl_2$ and containing an additional 2.2 mM $MgSO_4$. In the experiments assessing the effects of drugs on histamine-induced CA release, four incubations were used. The first two were 5 min washes, the third a 5 min preincubation in the presence of drug, and the fourth a 20 min stimulation period with histamine in the presence of drug.

The medium from the third (and, where appropriate, the fourth) incubation periods was collected into perchloric acid (PCA, 0.4 M final concentration) and assayed for adrenaline (Ad) and noradrenaline (NA) by separation on a reverse-phase h.p.l.c. column and quantified by electrochemical oxidation (for full details see Livett *et al.*, 1986a; Marley *et al.*, 1986a,b). Cellular CA were extracted from the cultured cells in 0.01 M PCA, after their final incubation, and protein precipitated by acidifying to 0.4 M PCA. The release of each CA is expressed as a fraction (%) of its intracellular content as calculated immediately before the stimulation period. The two opioid peptides used in this study were dissolved in 0.1 M sodium phosphate, pH 7.1, and the concentrations of these stock solutions determined from their absorption at 280 nm assuming a molar extinction coefficient for tyrosine in this buffer of 1280.

Drugs

Dynorphin-1-13 was from Peninsula Laboratories (San Carlos, CA, U.S.A.). Metorphamide was a gift from Dr E. Weber (Stanford, CA, U.S.A.). Diprenorphine HCl was a gift from Mr I. Mawhinney (C-Vet Ltd., U.K.). Morphine HCl was from Prosana Laboratories (Sydney, Australia). Mepyramine maleate, cimetidine and hexamethonium bromide were from Sigma Chemical Co. (St. Louis, U.S.A.).

Results

Histamine-induced catecholamine secretion

Histamine produced a dose-dependent release of both Ad and NA from the chromaffin cells with a threshold concentration of approximately 5 nM, a maximal effect at 10 μM and an EC_{50} of 150 nM (Figure 1). NA and Ad secretion were equally sensitive to histamine; however, the proportion of cell content of each amine released by a given histamine concentration varied between cell preparations. In the cell preparations used for Figures 1, 2 and 4d, 1 μM histamine induced proportionately more Ad secretion than NA, while in cell preparations used for Figures 4a and 4c, 1 μM histamine induced the same % release of Ad and NA.

Histamine-induced secretion of both Ad and NA from the cultured chromaffin cells was completely dependent on extracellular calcium ions. Replacement of $CaCl_2$ in the incubation medium with an equimolar amount of $MgSO_4$ reduced histamine-induced CA secretion to basal levels (Figure 2). The calcium-dependency of histamine-induced CA secretion was seen for histamine concentrations from 1 nM–100 μM (data not shown).

Mepyramine, a selective H_1 histamine-receptor antagonist, inhibited histamine-induced CA secretion in a dose-dependent manner (Figure 3). It showed no selectivity for inhibiting Ad versus NA secretion, and abolished 1 μM histamine-induced secretion of both CA at a concentration of 0.1–1 μM. A concentration of mepyramine (0.1 μM) that virtually abolished histamine-induced CA release from cultured chromaffin cells (Figure 4a) had no effect on nicotine-induced CA secretion from parallel cultures (Figure 4b). Histamine-induced CA release was unaffected by high doses of cimetidine (a selective H_2 histamine-receptor antagonist, Figure 4c) or of hexamethonium (Figure 4d).

Effects of opioid peptides and morphine on histamine-induced catecholamine secretion

In previous studies, it was found that C-terminally extended opioid peptides had relatively high affinity for bovine adrenal medullary opioid binding sites, as determined from ligand-binding experiments (Castanas *et al.*, 1985a,b). The larger opioids were also found to be considerably more potent than the penta- and hexa-peptides at inhibiting the nicotinic secretory response of bovine chromaffin cells (Dean *et al.*, 1982; Marley *et al.*, 1986a).

In the present study, therefore, two C-terminally extended opioid peptides, dynorphin-1-13 and metorphamide, were tested for their ability to modify histamine-induced CA secretion from the cultured chromaffin cells. These two peptides were the most

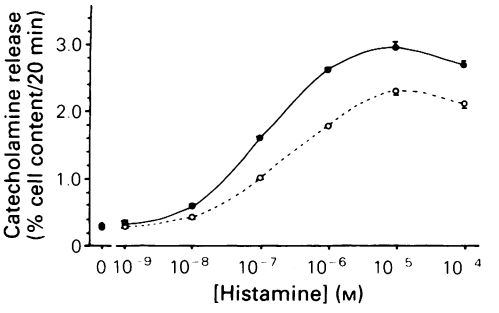

Figure 1 Concentration-dependence of histamine-induced adrenaline (●) and noradrenaline (○) secretion from monolayer cultures of bovine, isolated, adrenal chromaffin cells. Results are mean for $n = 4$ (s.e.mean shown by vertical line), and are representative of similar data from two cell preparations.

potent of 21 opioid peptides tested on the nicotinic response (Marley *et al.*, 1986a,b) and are both endogenous to the bovine adrenal medulla (Denis *et al.*, 1982; Weber *et al.*, 1983). Dynorphin-1-13, in the concentration range 1 nM–20 μM, and metorphamide, in the range 0.1 nM–10 μM, had no effect on the secretion of Ad or NA from cultured bovine chromaffin cells induced by 1 μM histamine (Figures 5a and 5b).

The ligand binding studies of Castanas *et al.* (1985a,b), have shown that dynorphin-1-13 has a relatively high affinity for bovine adrenal medullary κ_1

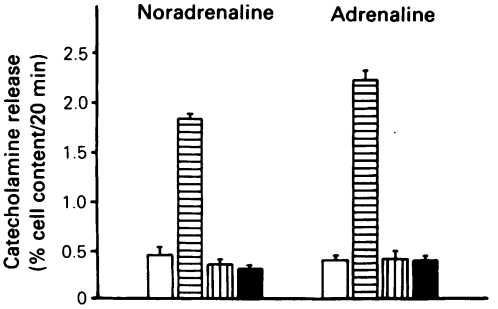

Figure 2 Calcium-dependence of 1 μM histamine-induced adrenaline and noradrenaline secretion from monolayer cultures of bovine, isolated, adrenal chromaffin cells. Solutions from which $CaCl_2$ was omitted contained an additional 2.2 mM $MgSO_4$. Open columns: basal secretion; horizontally hatched columns: effect of histamine 1 μM; vertically hatched columns: zero Ca^{2+} basal secretion; solid columns: effect of zero Ca^{2+} plus histamine 1 μM. Results are mean for $n = 10$–14; s.e.mean shown by vertical lines.

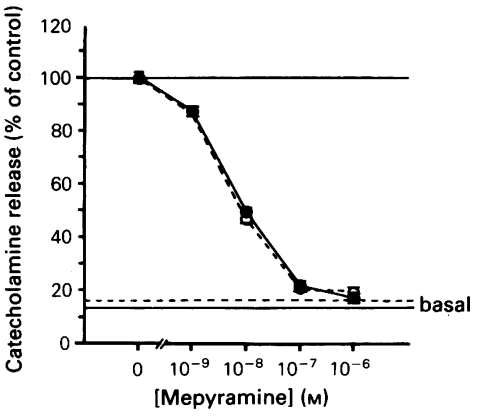

Figure 3 Inhibition by mepyramine, an H_1-receptor antagonist, of 1 μM histamine-induced adrenaline (Ad) (●) and noradrenaline (NA) (○) secretion from bovine cultured, adrenal chromaffin cells. Results are mean for $n = 4-8$; s.e.mean shown by vertical lines. In the absence of mepyramine, 1 μM histamine-induced the secretion of 3.35 (±0.06)% cell content of NA per 20 min and 2.49 (±0.03)% cell content Ad per 20 min.

and δ opioid binding sites, has lower affinity for μ sites and is very poorly recognised by κ_2 and κ_3 sites. Metorphamide however, exhibits high affinity for μ and δ sites (studied with guinea-pig brain membranes) and for κ sites characterized with [^{3}H]-bremazocine (Weber *et al.*, 1983). Its affinity for the three types of κ site identified in bovine adrenal medullary tissue by Castanas *et al.* (1985a,b) is not known. It was possible therefore that κ_2 or κ_3 opioid recognition sites on the adrenal chromaffin cells might modify histamine-induced CA release but that neither dynorphin-1-13 nor metorphamide might exhibit this action due to their low affinity for these receptors.

Consequently, morphine was tested for its ability to modify histamine-induced CA release from the cultured bovine chromaffin cells. Morphine has been shown to have high affinity for bovine adrenal medullary μ sites, to have slightly lower affinity for κ_1 sites, and to have fairly low but readily measured affinity for the δ, κ_2 and κ_3 sites (Castanas *et al.*, 1985a,b). However, morphine in the concentration range of 1 nM–0.1 mM had no effect on histamine-induced secretion of chromaffin cell CA (Figure 5c).

Since opioid peptides are present in bovine adrenal chromaffin cells in very high concentrations (see Marley & Livett, 1985) and are secreted along with CA upon stimulation of the medullary cells with appropriate stimuli (Livett *et al.*, 1981; Rossier *et al.*, 1981), it is possible that histamine may also induce opioid peptide secretion from the cultured chromaffin cells during CA release. Consequently, any opioid receptors the chromaffin cells possess may be fully occupied and activated during histamine-induced CA release by these secreted endogenous peptides. This may mask any effect of exogenous opioid agonists.

To test this possibility, the ability of diprenorphine to modify histamine-induced CA release was assessed. Diprenorphine is a potent opioid-receptor antagonist with very high affinity for all the opioid binding sites characterized in the bovine adrenal medulla (Castanas *et al.*, 1985a,b). However in concentrations of 1 nM–0.1 mM, diprenorphine failed to affect CA secretion induced by histamine (Figure 5d).

Discussion

Histamine-induced catecholamine secretion

The ability of histamine to induce adrenal CA release is an important physiological mechanism during anaphylactic shock to counter the hypotensive and bronchoconstrictor effects of histamine (see Cession-Fossion & Lecomte 1966; Lish *et al.*, 1966). Histamine has been known for many years to be capable of inducing secretion of adrenomedullary catecholamines when given to cats or dogs intravenously (Elliot, 1912; Szczygielski, 1932; Wada *et al.*, 1940). In dogs, this action of histamine is completely abolished by transection of the splanchnic nerves or by ganglionic blockade, suggesting it is mediated indirectly by a central reflex mechanism (see Wada *et al.*, 1940; Staszewska-Barczak & Vane, 1965) or that histamine in some way potentiates the response of the adrenal medulla to neural drive. In the cat and the rat, however, splanchnicotomy and ganglionic blockade do not reduce the effect of histamine on adrenal CA release (Szczygielski, 1932; Slater & Dresel, 1952; Trendelenburg, 1954; Staszewska-Barczak & Vane, 1965; Cession-Fossion & Lecomte, 1966; Yoshizaki, 1973; Z. Khalil, Marley & Livett – unpublished observations). CA secretion could also be induced in the rat and the cat by injecting or infusing histamine directly into the adrenal gland either *in situ* or in the isolated perfused gland (Szczygielski, 1932; Yoshizaki, 1973), and this secretion was not prevented by anticholinergic drugs. These data suggest that, in the cat and rat, histamine has a direct action on the adrenal medulla to induce Ad and NA secretion, independently of the cholinergic innervation of the chromaffin cells.

This direct action of histamine is dependent on extracellular calcium (Poisner & Douglas, 1966 – cat), and is mediated by H_1 histamine-receptors (Emmelin & Muren, 1949; Trendelenburg 1954; Staszewska-Barczak & Vane, 1965; Yoshizaki, 1973; Robinson, 1982 – cat and rat). In the present study, identical characteristics have been found for histamine-induced CA release from monolayer cultures of bovine, isolated, adrenal

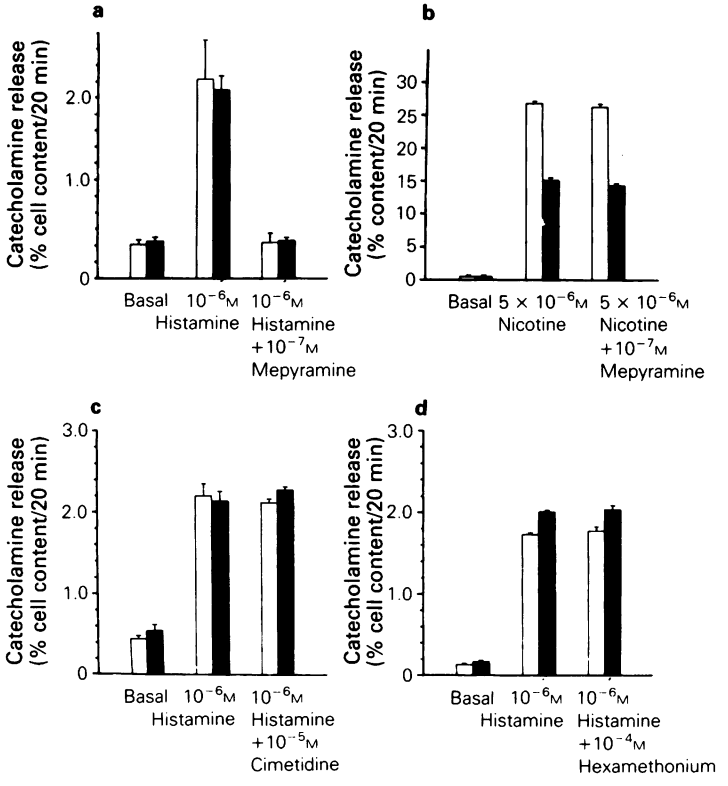

Figure 4 Pharmacology of 1 μM histamine-induced adrenaline (closed columns) and noradrenaline (open columns) secretion from bovine cultured adrenal chromaffin cells. (a) Effect of 0.1 μM mepyramine; (b) effect of 0.1 μM mepyramine on 5 μM nicotine-induced catecholamine release; (c) effect of 10 μM cimetidine; (d) effect of 0.1 mM hexamethonium. Results are mean for $n = 6-8$; s.e.mean shown by vertical lines. Note change in scale in (b).

chromaffin cells. Although histamine has been shown previously to induce CA release from bovine adrenal medulla (Schneider, 1969; Shima *et al.*, 1976), the pharmacology of this action has not been studied. On cultured bovine adrenal chromaffin cells, we have found that histamine induces both Ad and NA secretion in a concentration- and calcium-dependent manner, by an action on H_1-receptors, and independently of the nicotinic receptors (Figures 1–4). It was noteworthy that histamine had a much lower EC_{50} (150 nM) than nicotine (approximately 5 μM, see Marley *et al.*, 1986a), but had a much lower efficacy than nicotine (compare Figures 4a and b). Histamine also tended to induce proportionately slightly more Ad secretion than NA secretion (Figures 1, 2, 4) whilst nicotine induced proportionately twice as much NA secretion than A secretion (Figure 4b). Histamine when administered intravenously to anaesthetized animals has been reported to release Ad selectively from the intact adrenal gland of the cat (Staszewska-Barczak & Vane, 1965) the dog (Schaepdryver, 1959; Narita, 1971), and the rat (Z. Khalil, Marley & Livett – unpublished observations).

Physiologically, histamine may be released by mast cells during anaphylactic reactions and affect adrenal CA secretion via the circulation. However, histamine is also found within the adrenal gland itself (Endo & Ogwa, 1974) and has recently been localised by immunohistochemistry to the noradrenaline-containing chromaffin cells in the rat (Happola *et al.*, 1985). It is possible, therefore that histamine may play a local regulatory role within the adrenal medulla, as well as its better characterised actions on the adrenal during anaphylactic shock. The concentration of histamine used in this study (1 μM) is very comparable to that required to induce contractions of trachea or relaxation of peripheral smooth muscle (see, e.g., Suzuki *et al.*, 1983), and is some five fold higher than histamine concentrations in resting peripheral plasma (Man *et al.*, 1984).

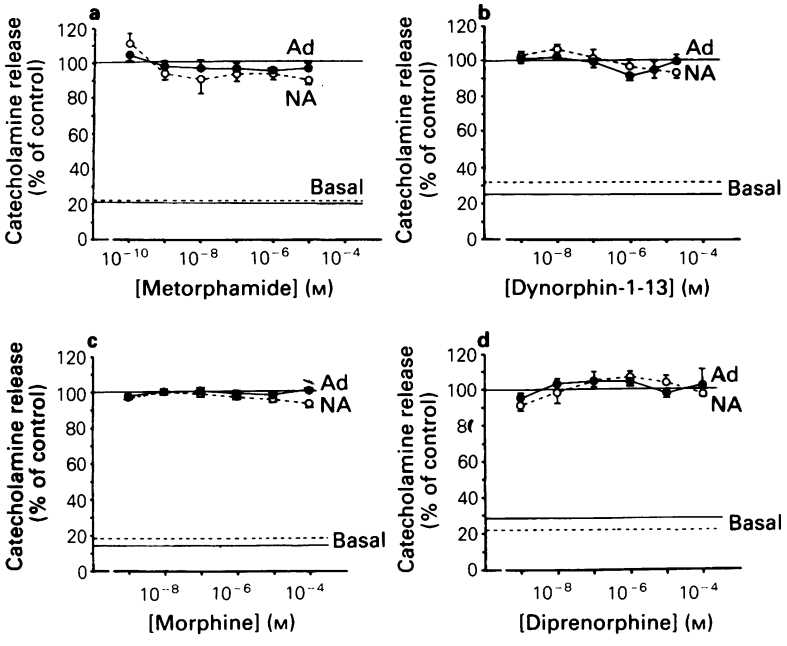

Figure 5 Lack of effect of opioid agonists and antagonists on 1 μM histamine-induced adrenaline (Ad) and noradrenaline (NA) secretion from bovine cultured, adrenal chromaffin cells. (a) Metorphamide, (b) dynorphin-1-13, (c) morphine, (d) diprenorphine. Results are mean of $n = 6$; s.e.mean shown by vertical lines. Control responses to 1 μM histamine were: (a) 1.23 (±0.07)% cell content NA per 20 min and 2.14 (±0.09)% cell content Ad per 20 min, $n = 12$; (b) 1.46 (±0.03)% NA per 20 min and 2.37 (±0.04)% Ad per 20 min, $n = 18$; (c) 3.28 (±0.05)% NA per 20 min and 2.49 (±0.03)% Ad per 20 min, $n = 18$; and (d) 1.26 (±0.09)% NA per 20 min and 1.88 (±0.05)% Ad per 20 min, $n = 18$.

Effects of opioid peptides and morphine on histamine-induced catecholamine release

Opioid peptides synthesized and stored in adrenal medullary chromaffin cells are secreted concomitantly with CA during stimulation of the medullary cells (see Introduction). Since the chromaffin cells themselves possess binding sites for opioid peptides (Kumokura *et al.*, 1980), these peptides may act to regulate adrenal medullary CA secretion evoked by appropriate physiological stimuli. Previous studies have suggested that they do not inhibit the nicotinic secretory response of these cells in a physiological manner, since this effect of the peptides is very weak and is not blocked by opioid antagonists. The current study shows that opioid peptides and morphine also do not modify histamine-induced CA secretion from adrenal medullary cells. Between them, the three opioid agonists studied can act on all the known opioid binding sites in the bovine adrenal medulla (Castanas *et al.*, 1985a,b). Whether these opioid receptors on adrenal chromaffin cells can modify CA secretion induced by secretogogues other than those acting on histamine or nicotinic receptors remains to be determined.

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