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Role of μ-opioid receptors in insulin release in the presence of inhibitory and excitatory secretagogues

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Abstract

In mouse pancreatic islets incubated under static conditions, the inhibitory effects on glucose-evoked insulin release induced by adrenaline (1 μM), clonidine (2 μM) and UK 14,304 (brimonidine, 0.001–1 μM) were abolished by naloxone (30 nM). Only CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Phe-Thr-NH_2, 0.1 μM), a very selective μ-opioid receptor antagonist, blocked the response to UK 14,304. Glucose-induced insulin secretion was attenuated by both β-endorphin (0.01 μM) and endomorphin-1 (0.1 μM). Naloxone and CTOP prevented these inhibitory responses. The stimulatory effect of glibenclamide (1 μM) was also reduced by endomorphin-1. However, when islets were incubated in the presence of K^+ (30 mM), carbachol (100 μM) or forskolin (0.1 μM), neither the inhibitory effect induced by UK 14,304 was reversed by naloxone, nor endomorphin-1 altered the responses promoted by the excitatory agents. Thus, α_2-adrenoceptor stimulation might inhibit glucose-induced insulin secretion by releasing endogenous opioids. μ-Opioid receptor activation and opening of K_{ATP} channels could be involved in the response.

Keywords: α_2-Adrenoceptor stimulation; Opioid, endogenous; Insulin release; μ-Opioid receptor

1. Introduction

It is well established that stimulation of α_2-adrenoceptors mediates an inhibitory effect on insulin secretion. The molecular processes by which adrenaline and other α_2-adrenoceptor agonists lead to this inhibitory response include a number of mechanisms coupled to the G_i/G_o signalling system as: inhibition of adenyl cyclase and cAMP production (Nakaki et al., 1981), activation of K^+ channels (Drews et al., 1990; Rorsman et al., 1991) and inhibition of L-type voltage-dependent Ca^{2+} channels (Nilsson et al., 1988; Keahey et al., 1989). More recent work postulates a direct effect on the secretory machinery distal to Ca^{2+} influx (Sharp, 1996; Lehr et al., 1997), secondary to activation of the protein phosphatase calcineurin (Renström et al., 1996). This α_2-adrenoceptor seems to belong to the α_{2A} subtype (Niddam et al., 1990; Angel et al., 1992; Devedjian et al., 2000).

The involvement and specific interaction established between opioidergic and α_2-adrenergic mechanisms in a number of physiological responses such as antinociception (Mastrianni et al., 1989; Meert and De Kock, 1994; Park et al., 1996; Alberti et al., 1999), cardiovascular depression (Kunos et al., 1981; Mastrianni and Ingenito, 1987) and the increase in renal osmolar clearance (Intengan and Smyth, 1996, 1997) are also well known. It has also been suggested that opioids may participate in the effect of stress (catecholamines) on pancreatic islets (Giugliano et al., 1988a,b) and hormone release.

Considerable evidence indicates that endogeneous opioid peptides are expressed in endocrine pancreas (Cetin, 1990; Khawaja et al., 1990; Josefsen et al., 1998). Islet cell secretion of these peptides could influence β-cell function. A number of in vitro and in vivo studies have demonstrated direct effects of endogenous and selective opioid receptor agonists on insulin release (Giugliano et al., 1988b; Ahren, 1989). However, these observations have yielded conflicting results with marked variations between species (Josefsen et

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al., 1998). β-Endorphin seems to inhibit glucose-induced insulin secretion (Schleicher, 1989), but an excitatory effect was found after an i.v. bolus injection (Reid and Yen, 1981). Interestingly, the presence in the endocrine pancreas of μ-, δ- and κ-opiate binding sites has been described in a number of rodents (Khawaja et al., 1990; Cetin, 1990; Zhang et al., 1994). Therefore, the present work using mouse islets is mainly directed to analyse the involvement and nature of opioid receptors on the insulin inhibitory response derived from α_2-adrenoceptor stimulation when insulin release was elicited in the presence of glucose and a number of selective secretagogues.

2. Material and methods

2.1. Solutions

The medium used was a bicarbonate-buffered solution, which contained (mM) 120 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$ and 24 $NaHCO_3$. It was gassed with O_2/CO_2 (94:6) to maintain pH=7.4. In high K^+ solutions (KCl 30 mM), the concentration of NaCl was decreased to 95 mM. The solutions used were supplemented with 1 mg ml^{-1} bovine serum albumin fraction V (Boehringer Mannhein, Germany). The inhibitors of protein degradations (captopril, bestatin and DL-thiorphan, 0.01 μM) were added to the solution containing the opioid peptides.

2.2. Measurement of insulin secretion

All experiments were performed with islets isolated by collagenase digestion, followed by hand picking (Jonas et al., 1998) of the pancreas from fed female CD1 mice (25–30 g) killed by decapitation. After isolation, the islets were preincubated at 37 °C for 1 h in a control medium containing 15 mM glucose, a concentration that causes half-maximal stimulation of insulin secretion in mouse islets. The islets were then incubated for 60 min, in batches of three, in 1 ml of medium containing appropriate concentrations of the test substances. At the end of the incubation, a portion of the medium was withdrawn and diluted before insulin assay measurement was performed by double antibody radioimmunoassay (CIS Radioquímica-Schering, Spain).

2.3. Drugs

Naloxone hydrochloride was supplied by Abelló (Spain); adrenaline hydrochloride, 2-methoxyidazoxan hydrochloride, captopril, bestatin, DL-thiorphan, forskolin, galanine, adenosine, diazoxide, glibenclamide and carbamylcholine chloride (carbachol) were provided by Sigma Aldrich Quimica (Spain); clonidine hydrochloride was obtained from Boehringen Ingelheim (Germany); UK 14,304 (brimonidine) came from Pfizer (UK); CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Phe-Thr-NH_2) and β-endorphin were obtained from RBI (USA); endomorphin-1, ICI 174,864 (*N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu) and nor-binaltorphimine dihydrocloride were from Tocris (Bristol, UK).

2.4. Statistics and data analysis

Results are presented as means±S.E.M. for the indicated number of batches of islets from three to five separate experiments as specified. The statistical significance of differences between means was assessed by analysis of variance followed by a Newman–Keuls test for multiple comparisons. Differences were considered statistically significant if $P<0.05$.

3. Results

3.1. Effects on insulin release induced by α_2-adrenoceptor stimulation in the presence of naloxone or/and α-adrenoceptor antagonists

The insulin secretory response to 15 mM glucose was significantly reduced by UK 14,304 (brimonidine) in a dose-dependent manner (maximum degree of inhibition: 67.8±2.1%, n=14, $P<0.001$ was reached at 1 μM; Fig. 1A. No further reduction was found with higher concentrations). Interestingly, the opioid receptor antagonist, naloxone (30 nM), unable itself to alter glucose-induced insulin release, completely reversed or markedly attenuated the inhibitory responses elicited by the agonist (Fig. 1A). Similarly, naloxone prevented the well-established inhibitory effect mediated by either clonidine (2 μM) or adrenaline (1 μM; Fig. 1B). Percentage of inhibition in the presence of these agonists, respectively, was 32.6±2.5% (n=10, $P<0.05$) and 29.6±3.5% (n=12, $P<0.01$). Basal insulin release in the presence of 15 mM glucose ranged between 55.5 and 62.75 μIU/ml/islet/h.

As expected, the selective α_2-adrenoceptor antagonist methoxyidazoxan (0.1 μM) blocked the inhibitory responses mediated by UK 14,304 and clonidine. Once this inhibitory effect was prevented by the competitive antagonist, the administration of naloxone did not evoke any further alteration on insulin release (data not included). Reversal by methoxyidazoxan and naloxone of the inhibitory effect induced by UK 14,304 on glucose-induced insulin secretion was explored in more detail when islets were incubated in the presence of a high concentration of the nutrient (30 mM), the α_2-adrenoceptor agonist (1 μM) and progressive increasing concentrations of either antagonist (Fig. 2). Methoxyidazoxan, but not naloxone, behaved as a classical competitive antagonist. In the presence of the opioid receptor antagonist, a similar degree of recovery was found between 0.3 and 300 nM. Basal insulin release in the presence of 30 mM glucose fluctuated between 81.2 and 87.3 μIU/ml/islet/h.

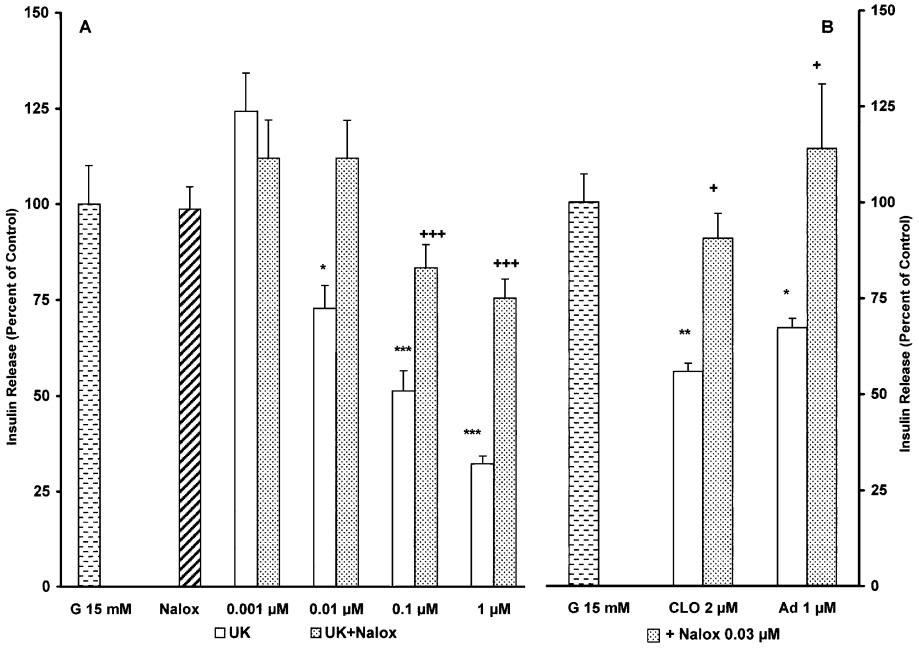

Fig. 1. Effects of adrenaline (Ad), clonidine (CLO) and UK 14,304 (brimonidine or UK) on insulin release in the absence and presence of naloxone (30 nM, ▨). The effect of the opioid antagonist alone is also presented (A). Mouse islets were incubated for 1 h in 1 ml of medium containing the indicated drug concentrations in 15 mM glucose. Values are expressed as percentages of mean control (100%)±S.E.M. for 20 batches of islets from four experiments. *P<0.05, **P<0.01, ***P<0.001, values significantly different from the insulin secretory response to glucose. ^{+}P<0.05, ^{+++}P<0.001, when the level of significance was established in the presence of naloxone.

3.2. Inhibition by UK 14,304 of glucose-induced insulin secretion in the presence of selective opioid receptor antagonists

Since naloxone has the ability to block the three different types of opioid receptors, it was mandatory to test the effect of CTOP, nor-binaltorphimine and ICI 174.864, selective antagonists for μ-, κ- and δ-opioid receptors, respectively. Only CTOP (0.1 and 1 μM) antagonised the inhibitory response mediated by UK 14,304. Neither nor-binaltorphimine (0.1 and 1 μM) nor ICI 174.864 (0.1 and 1 μM)

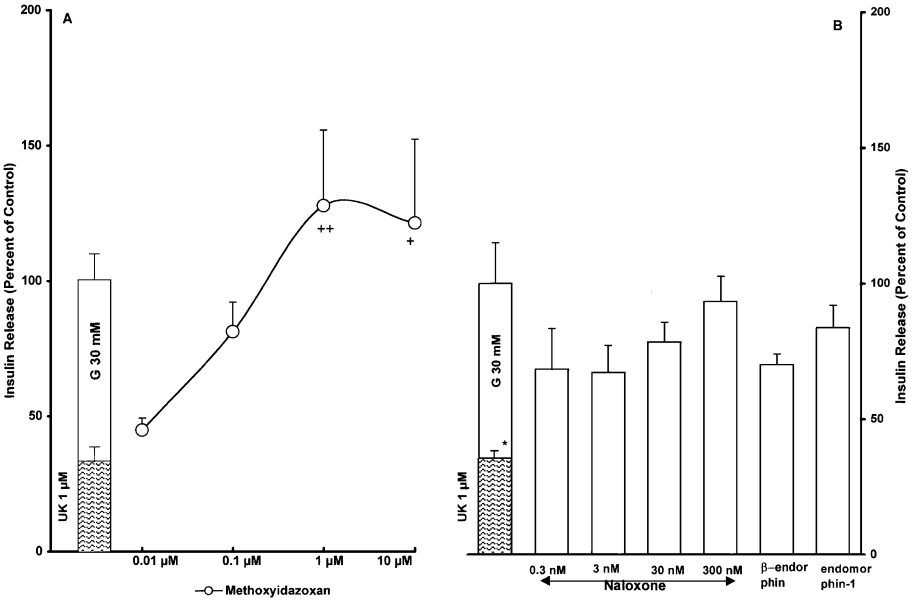

Fig. 2. Concentration dependency of the effects of methoxyidazoxan (A) or naloxone (B) on insulin release by incubated mouse islets. The incubation medium contained 30 mM glucose (open column) in the presence of UK 14,304 (hatched column). Results are expressed as a percentage of insulin release in the presence of glucose and the α_2-adrenoceptor agonists. ^{+}P<0.05, ^{++}P<0.01, when the level of significance is established between antagonist-treated and nontreated islets. The effects of both endogenous opioid receptor agonists, β-endorphin (0.01 μM) and endomorphin-1 (0.1 μM) when islets are incubated in 30 mM glucose alone are also presented in (B). *P<0.05. For more details, see legend of Fig. 1.

modified the response derived from α_2-adrenoceptor stimulation (Fig. 3A). Glucose-induced insulin release was not altered by the addition of any of the opioid receptor antagonist, regardless of the added concentration.

3.3. Inhibition of glucose-induced insulin release by other selective agonists in the presence of naloxone

Three agonists with a well-known ability to inhibit insulin release, the peptide galanin (Lindskog and Ahren, 1991), adenosine (Bertrand et al., 1989) and the ATP-sensitive potassium channel opener diazoxide (Jonas et al., 1994) were chosen for this study. Insulin secretion in the presence of 15 mM glucose was significantly reduced by 0.05 μM galanine (percent inhibition=38.5±5.0%, n=20, P<0.001), 500 μM adenosine (42.0±2.4%, n=14, P<0.001) and 40 μM diazoxide (37.5±2.6%, n=20, P<0.001). The inclusion of naloxone (30 nM) did not modify the inhibitory responses evoked by either agonist (Fig. 3B).

3.4. Effects of endogenous opioid receptor agonists on glucose-induced insulin secretion

Since μ-opioid receptors seem to be involved in the inhibitory response derived from α_2-adrenoceptor stimulation, two selective endogenous μ-opioid receptor agonists (β-endorphin and endomorphin-1) were assayed against glucose. As previously described, β-endorphin induced a biphasic effect: inhibition of glucose-induced insulin release at a low concentration (0.01 μM 31.3±5.3%, n=14, P<0.05), and an increase in hormone secretion with higher doses (1 μM). The inhibitory response was suppressed by the addition of naloxone (Fig. 4A). Endomorphin-1 also reduced the response to glucose. However, a dose-related effect was not found (Fig. 4B) since a similar degree of inhibition was induced by either 0.01–0.1 μM. (maximum degree of inhibition being 37.0±6.2%, n=28, P<0.01; lower concentrations were ineffective). While CTOP (0.1 μM) neutralised the agonist response, methoxyidazoxan (0.1 μM) did not (Fig. 4C). Interestingly, endomorphin-1 also attenuated the insulin secretory response evoked by glibenclamide, 1 μM (increase in insulin release in the absence and presence of the peptide being 163.3±14%, n=12; 108.5±14.3%, n=12, P<0.01; Fig. 4C).

When islets were incubated in the presence of a higher glucose concentration (30 mM), both endogenous opioid receptor agonists significantly reduced insulin release (percent inhibition with endomorphin-1 and β-endorphin being, respectively, 30.0±3.9%, n=10, P<0.01; 15.3±8.2%, n=12, P<0.05; Fig. 2B). The inhibitors of protein degradation did not modify the response to glucose (data not included).

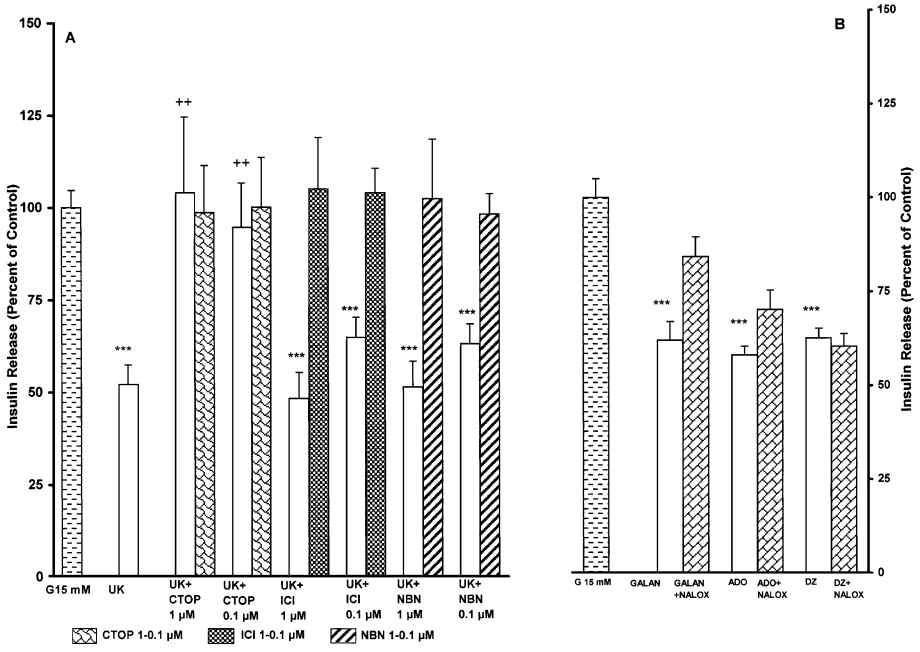

Fig. 3. (A) Effects of UK 14,304 (UK, 1 μM) on insulin release in the absence and presence of CTOP (1 and 0.1 μM), ICI 174.864 (1 and 0.1 μM) and norbinaltorphirmine (NBN, 1 and 0.1 μM). The effects of the selective antagonists by themselves on insulin release are also shown. (B) Inhibitory effects on glucose-induced insulin release by galanine (GALAN, 0.05 μM), adenosine (ADO, 500 μM) and diazoxide (DZ, 40 μM) in the absence or presence of naloxone (NALOX, 30 nM). **P<0.01, ***P<0.001, values significantly different from the insulin secretory response to glucose. ^{++}P<0.01 when the level of significance was established between UK 14,304 vs. UK+CTOP-treated islets.

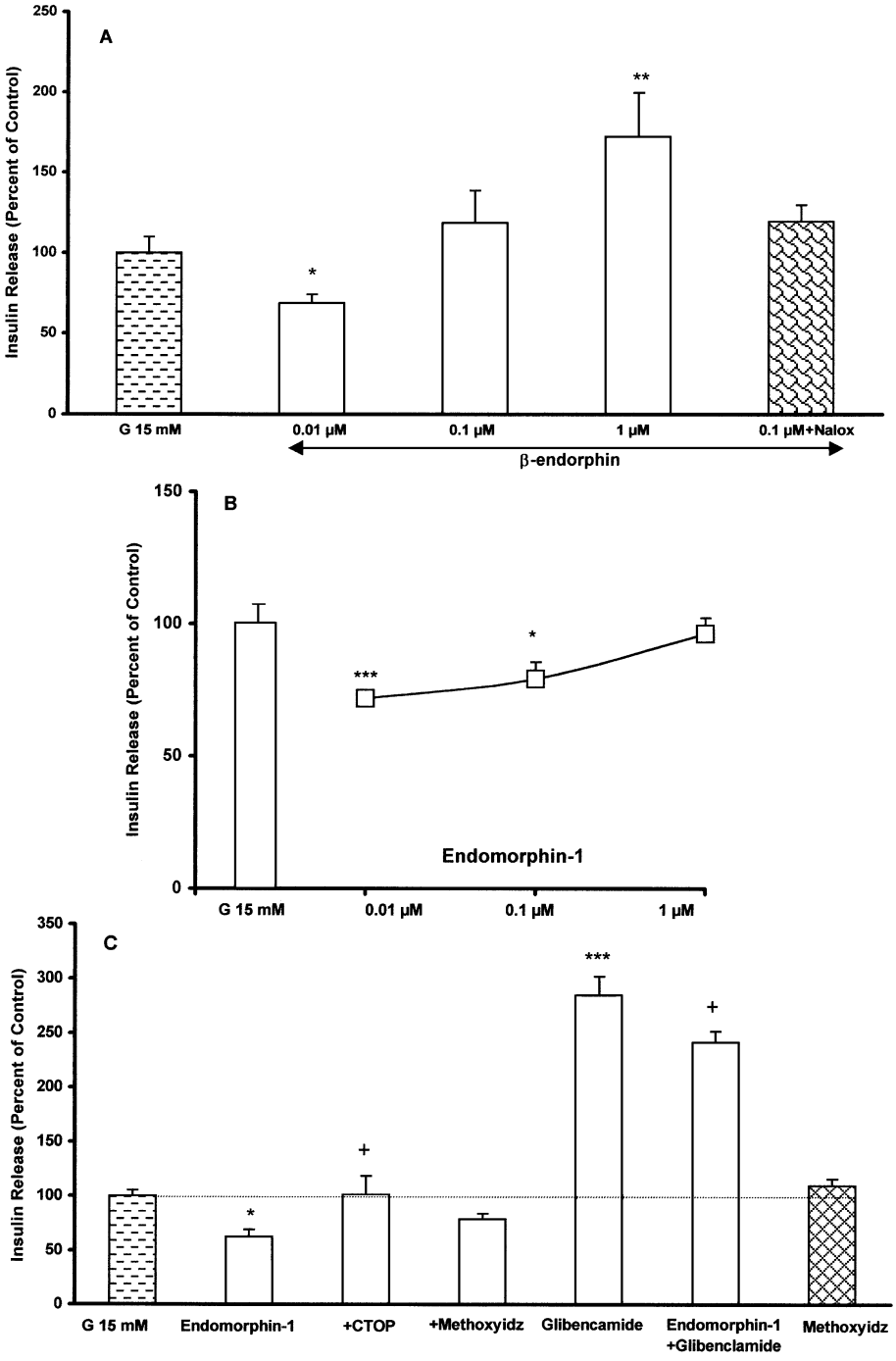

Fig. 4. The effects of increasing concentration of β-endorphin (A) and endomorphin-1 (B) on glucose-induced insulin release. The inhibitory responses mediated by the endogenous opioids in the presence of the antagonists (A and C), as well as the excitatory effect of glibenclamide (1 μM) in the absence and presence or endomorphin-1 (0.1 μM) are also shown (C). *P<0.05, **P<0.01, ***P<0.001, vs. glucose alone. ^{+}P<0.05 vs. agonist- and glibenclamide-treated islets. For more details, see legends of Figs. 1 and 2.

3.5. Effect of UK 14,304 on the insulin secretory responses induced by forskolin, carbachol and K^+: role of naloxone

In the presence of 15 mM glucose, 0.1 μM forskolin induced a remarkable increase in insulin release (Δ=350.6±51.3%, n=15, P<0.001), response which was severely depressed by UK 14,304 (Δ=−45.0±7.5%, n=15, P<0.001; Fig. 5). Naloxone was unable to reverse the inhibitory effect mediated by the α_2-adrenoceptor agonist and the endogenous opioid receptor agonist endomorphin-1 (0.1 μM) did not modify the response to forskolin (Fig. 5).

The inclusion of carbachol (100 μM) in a medium containing 15 mM glucose elicited a very severe increase in insulin release (Δ=195.9±41%, n=25, P<0.001), which

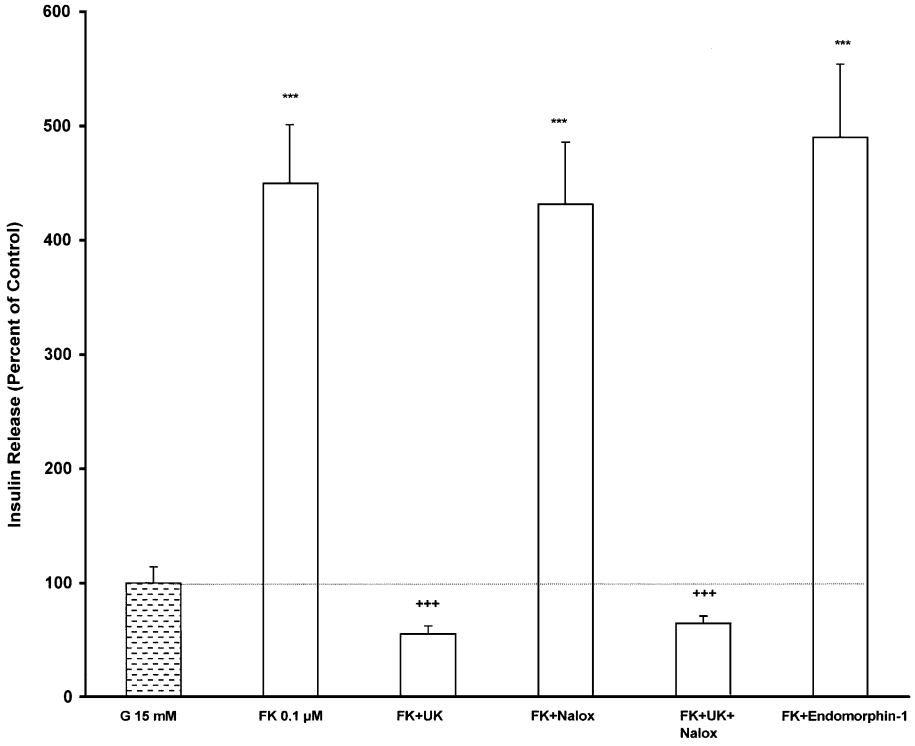

Fig. 5. Effects of forskolin (0.1 μM) on 15 mM glucose-induced insulin release from mouse isolated islets, in the absence or presence of naloxone (30 nM) and endomorphin-1 (0.1 μM). ***P<0.001 vs. glucose alone. The inhibitory effect of UK 14,304 (1 μM) on forskolin-induced insulin release in the absence or presence of naloxone is also presented. ^{+++}P<0.001 vs. forskolin-treated islets.

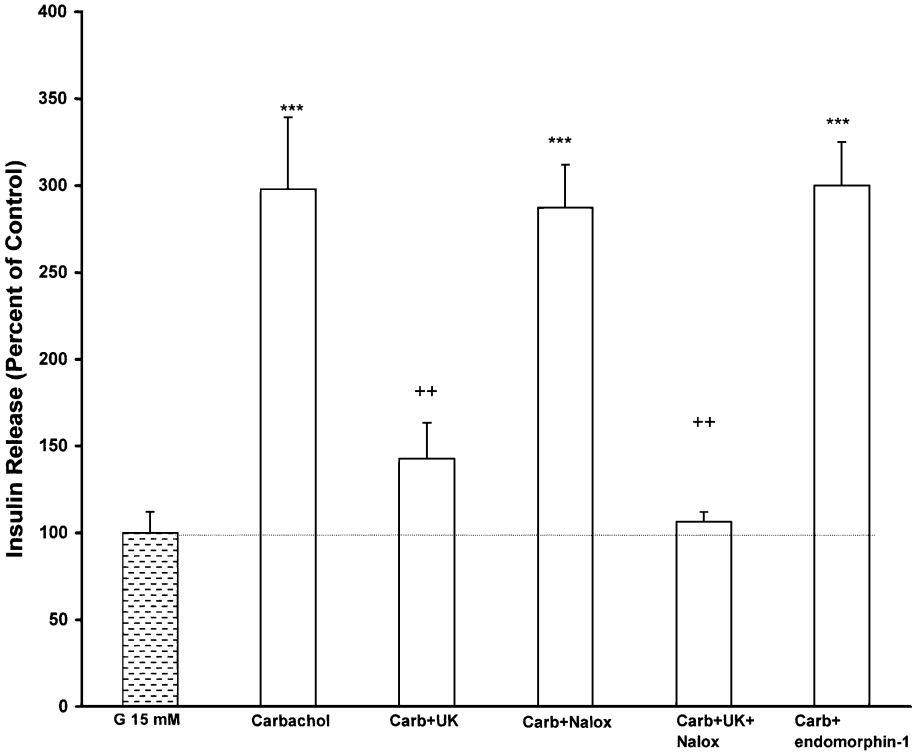

Fig. 6. Effects of carbachol (100 μM) on 15 mM glucose-induced insulin release from mouse isolated islets, in absence and presence of naloxone (30 nM) or endomorphin-1 (0.1 μM). ***P<0.001 vs. glucose alone. Inhibition of carbachol-mediated insulin release by UK 14,304 (UK 1 μM) in the absence or presence of naloxone is also presented. ^{++}P<0.01, ^{+++}P<0.001, vs. carbachol-treated islets.

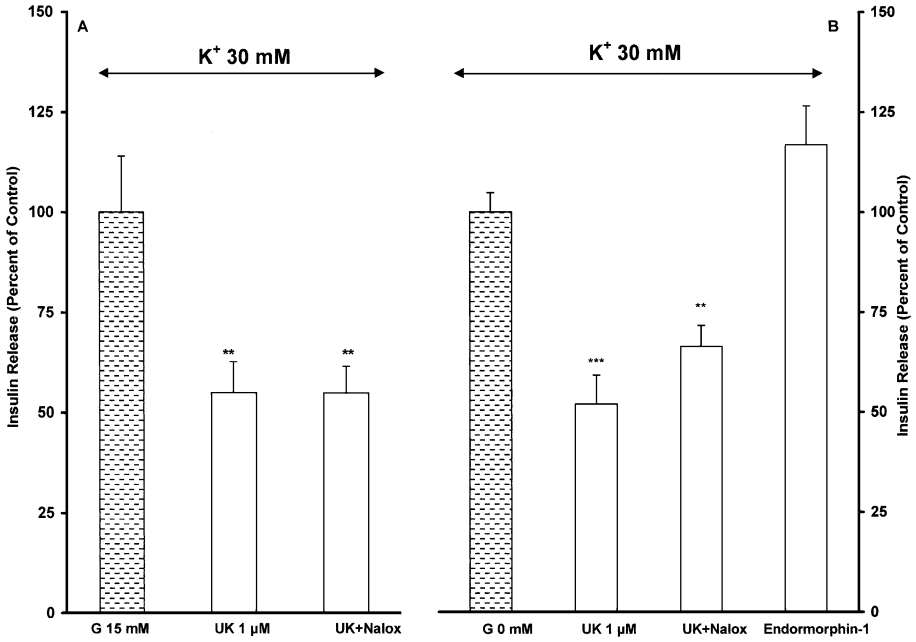

Fig. 7. (A) Effect of UK 14,304 (1 μM) on insulin release in the absence and presence of naloxone (NALOX, 30 nM) in a medium containing 30 mM $K^+$and 15 mM glucose. (B) Effect of UK 14,304 (1 μM) on insulin release in the absence and presence of naloxone or endomorphin-1 (0.1 μM) in a glucose-free medium containing 30 mM K^+. Values indicate mean±S.E.M. for 24 batches of islets from five experiments. **P<0.01, ***P<0.001 (significance level vs. glucose).

was significantly attenuated by the presence of the α_2-adrenoceptor agonist (1 μM; Δ=42.6±21.6%, n=26, P<0.001 when compared to carbachol alone). However, inhibition of carbachol-evoked insulin release by UK 14,304 was not modified by naloxone (Δ=49.4±16.1%, n=22, n.s. when compared to carbachol+UK 14,304; Fig. 6). Interestingly, endomorphin-1 did not alter the excitatory response induced by carbachol.

Finally, when the insulin secretory response was explored increasing the extracellular concentration of K^+ (30 mM), either in the presence of glucose (15 mM) or in a glucose-free medium, the inclusion of UK 14,304 (1 μM) significantly attenuated the response in both experimental conditions (percent inhibition=49.0±7.1%, n=20, P<0.01; 48.0±7.3%, n=20, P<0.01, in glucose-rich and glucose-free media, respectively; Fig. 7). Regardless the presence or absence of glucose, the inhibitory effect mediated by the α_2-adrenoceptor agonist remained the same in the presence of naloxone. Interestingly, endomorphin-1 (0.1 μM) failed to reduce the insulin secretory response elicited by K^+ (K^+-induced insulin release in 15 mM glucose and glucose-free, respectively, was: 92.5±13.1 μIU/ml/islet/h, n=18; 45.8±2.2 μIU/ml/islet/h, n=20).

4. Discussion

The present work clearly shows that, in mouse isolated islets, the inhibition of glucose-induced insulin release by adrenaline, clonidine and UK 14,304 is a naloxone-sensitive effect. A similar type of interaction between α_2-adrenoceptor agonists and opioid receptor antagonists has been described in other central and peripheral responses. Thus, the analgesic effect of systemically administered clonidine is inhibited by naloxone (Tchakarov et al., 1985; Nakamura and Ferreira, 1988; Mastrianni et al., 1989) or naltrindole (Alberti et al., 1999). Naloxone reverses the hypotensive effect of clonidine in hypertensive rats (Farsang and Kunos, 1979; Kunos et al., 1981; Mastrianni and Ingenito, 1987), and the increase in osmolar clearance induced by clonidine, guanfacine and UK 14,304 is likewise attenuated by naltrexone (Intengan and Smyth 1996, 1997). Recently, an interaction between I_2-imidazoline and opioid receptors mediating supraspinal analgesia in mice has also been reported (Sanchez-Blázquez et al., 2000). However, we believe that in our system, the effect on insulin release is dependent of α_2-adrenoceptor stimulation since the inhibitory (not an excitatory) response is mediated not only by clonidine nor UK 14,304 but even by adrenaline. Similarly, the selective α_2-adrenoceptor antagonist methoxyidazoxan blocked the inhibitory responses of both clonidine and UK 14,304.

On the other hand, reversal by naloxone of the inhibitory effect induced by α_2-adrenoceptor stimulation seems to result from the interaction of the opioid receptor antagonist with μ or μ-like receptors, since only the very selective antagonist CTOP (Gulya et al., 1988) abolished the response in the presence of UK 14,304, while nor-binaltorphimine and ICI 174,864, selective antagonists for κ- and δ-opioid receptors, respectively (Portoghese et al., 1987), were ineffective. The presence of μ-opioid receptors mediating the response described in our system was corroborated by studies using selective agonists (Zadina et al., 1997). In

fact, the administration of either β-endorphin or endomorphin-1 inhibited glucose-induced insulin release, an effect clearly abolished by naloxone or CTOP, being methoxyidazoxan-ineffective.

This naloxone/CTOP-sensitive response seems to be selectively linked to an α_2-adrenoceptor stimulation, since naloxone failed to antagonise the inhibitory effect on glucose-induced insulin release mediated by well-known agonists like galanine, adenosine and diazoxide.

A competitive and direct interaction of naloxone with α_2-adrenoceptors seems improbable, considering that the opioid receptor antagonist was unable to reverse the effect of UK 14,304 when insulin release was evoked by either carbachol, high concentrations of K^+ and forskolin. Even more, when the inhibitory effect of UK 14,304 was assayed in the presence of high glucose concentration, methoxyidazoxan and naloxone exhibited different behaviour. The classical pattern of competitive antagonism was found with the first, whereas a similar degree of recovery was described with naloxone, whatever the concentration used. It is also difficult to accept a non-selective post-receptor effect of naloxone considering our results in islets after the addition of other selective inhibitory agonists like galanine, adenosine and diazoxide.

Our results leave opened the possibility that α_2-adrenoceptor stimulation might inhibit glucose-induced insulin secretion through an indirect way by releasing endogenous opioids with affinity for μ-opioid receptors. Considerable evidence indicates that the endogenous opioids peptides and their receptors are expressed in the endocrine pancreas (Timmers et al., 1986; Josefsen et al., 1998). However, dynorphin-A increases glucose-induced insulin release (Green et al., 1983; Green and Tadayyon, 1988), whereas very conflicting data have been reported with Met-enkephalin (Hermansen, 1983; Giugliano et al., 1987). There is a general consensus that low doses of β-endorphin inhibit insulin release (Giugliano et al., 1988a; Zhang et al., 1994), an effect mediated by stimulation of either μ- or δ-opioid receptors (Zhang et al., 1994). In our system, μ-opioid receptors seem to mediate the opioidergic responses under study; the effect of β-endorphin was reproduced by the very selective μ-opioid receptor agonist endomorphin-1 (Zadina et al., 1997), and low doses of naloxone and CTOP blocked the effect. Consequently, the present work could well suggest that in the presence of glucose, α_2-adrenoceptor stimulation would inhibit insulin secretion by releasing either β-endorphin or endomorphin-1. (endomorphin-2, in the concentration range 0.001 – 0.1 μM, did not modify glucose-induced insulin release (data not included)). β-endorphin release after clonidine administration has already been reported (Mastrianni et al., 1989) and an increase in plasma β-endorphin levels with suppression of insulin release is associated with stressful situations (Giugliano et al., 1988a). Thus, endogenous opioids released by the β-cell could exert its effect either directly or through the paracrine system (Zhang et al., 1994).

Since endomorphin-1 is also able to attenuate the insulin secretory response induced by glibenclamide, we can postulate that the activation of K_{ATP} channels might mediate this inhibitory effect. It is known that sulphonylureas antagonize the antinociceptive effects of opioids (Ocaña et al., 1990), and that the peripheral analgesic effect induced by morphine is associated with ATP-sensitive K^+ channels (Rodrigues and Duarte, 2000). In keeping with this, it is also interesting to mention that activation of K^+ channels represents an important step in the transduction mechanism underlying the analgesia derived from α_2-adrenoceptor stimulation (Galeotti et al., 1999), and that stimulation by μ-opioid receptors and α_2-adrenoceptor share common effector mechanism in the central nervous system (Aghajanian and Wang, 1987).

However, when insulin release was stimulated either by forskolin, carbachol or high K^+ concentrations, the inhibitory effect induced by UK 14,304 was not reversed by naloxone. Interestingly, endomorphin-1 by itself did not reduce either the response evoked by the same agonists. The absence of a naloxone-sensitive effect would result from either the inability of UK 14,304 to release endogenous opioids in these experimental conditions or a failure of the own endogenous ligands to attenuate the response. We cannot rule out the first possibility, but our data in the presence of exogenous administered endomorphin-1 could well support the last one.

Though endomorphin-1 is considered as a very selective ligand for μ-opioid receptors, it could behave as a partial agonist. Various studies have demonstrated partial agonist activity of endomorphins (Harrison et al., 1998; Sim et al., 1998; Hososhata et al., 1998; Alt et al., 1998; Connor et al., 1999). Therefore, their weak efficacy helps to explain the inability of these peptides against agonists to stimulate insulin release as a result of the activation of complex and powerful signalling mechanisms. In this context, it is appropriate to quote that even a full agonist of K_{ATP} channels like diazoxide is unable to suppress the response to high K^+ (Gembal et al., 1993). In marked contrast with endogenous opioids, α_2-adrenoceptor agonists suppress insulin secretion regardless of the nature of the excitatory stimulus.

Summarizing, α_2-adrenoceptor stimulation might inhibit insulin secretion by an indirect mechanism in the presence of glucose: the release of endogenous opioids with affinity for μ-opioid receptors. When other agonists (forskolin, carbachol) are added, or in the presence of severe depolarisation (high concentration of extracellular K^+), the inhibitory adrenergic effect would be rather the result of a direct action on the exocytotic machinery, probably by activation of the protein phosphatase calcineurin (Renström et al., 1996).

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