Activation of protein kinase C partially alleviates noradrenaline inhibition of insulin secretion

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INTRODUCTION

In recent years many studies have attempted to define the mechanisms by which catecholamines inhibit secretion from pancreate B-cells, but the precise intracellular signalling pathways with which $\alpha_2$-adrenergic receptors interact are still unclear. Catecholamines are now known to act at several distinct sites within B-cells: early events include the inhibition of glucose metabolism (Laychock and Bilgin, 1987), inhibition of glucose-induced electrical activity (Joffre and Debyuser, 1990), thought to be secondary to activation of low-conductance K$^+$ channels (Rorsman et al., 1991), and changes in membrane fluxes of K$^+$ and Ca$^{2+}$ (Bertrand et al., 1989). $\alpha_2$-Adrenergic agonists also inhibit adenylate cyclase activity of islet homogenates (Howell and Montague, 1973; Kuo et al., 1973), and the resultant decrease in cyclic AMP generation in islets was proposed to mediate the inhibitory effects of catecholamines (Yamazaki et al., 1982). More recent studies have indicated that elevations of cyclic AMP alleviate, but do not abolish, the inhibition of insulin secretion by noradrenaline (NA) (Morgan and Montague, 1985; Tamagawa et al., 1985; Jones et al., 1987). Another major signalling pathway within B-cells is the phospholipase C-mediated hydrolysis of inositol phospholipids, by which inositol phosphates and diacylglycerol (DAG) are generated (Prenkt and Matschinsky, 1987). Catecholamines are without effect on B-cell inositol phospholipid turnover (Axen et al., 1983; Montague et al., 1985; Blachier et al., 1987), but to our knowledge there have been no reports on the ability of the DAG/protein kinase C (PKC) signal-transduction system to affect the inhibitory capacity of $\alpha_2$ agonists. In the present studies we have therefore assessed the effect of PKC activation on NA inhibition of insulin secretion, and studied the possible interactions between the stimulatory pathways activated by PKC and the inhibitory ones through which NA acts.

The sympathetic neurotransmitter noradrenaline (NA) fully inhibited both phases of glucose-stimulated insulin secretion from rat islets of Langerhans. The secretory response to the protein kinase C (PKC) activator, 4b-phorbol myristate acetate (4bPMA), in the absence of exogenous glucose was also abolished by NA. However, at 20 mM glucose 4bPMA partially alleviated the inhibitory effect of NA both on insulin release and on cyclic AMP generation. Inhibition of insulin release by NA, albeit much decreased, was still observed in the presence of maximal stimulatory concentrations of both 4bPMA and dibutyryl cyclic AMP. The relieving effect of 4bPMA on the inhibition of insulin secretion by NA was not overcome by the competitive antagonist of cyclic AMP-dependent protein kinase, Rp-adenosine 3',5'-cyclic phosphorothioate. Down-regulation of islet PKC activity by overnight exposure to 4bPMA did not affect the inhibitory capacity of NA. These results suggest that NA inhibits insulin release independently of interaction with PKC, but that activation of this enzyme decreases the inhibitory effect of NA at stimulatory concentrations of glucose. This protective effect of 4bPMA could not be attributed to a decrease in NA inhibition of cyclic AMP generation.

MATERIALS AND METHODS

Materials

Collagenase (type XI), BSA (fraction V), NA, ascorbic acid, dibutyryl cyclic AMP (db cAMP), 4b-phorbol myristate acetate (4bPMA), 4a-phorbol myristate acetate (4aPMA) and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma Chemical Co. (Poole, Dorset, U.K.). Na$^{125}$I for insulin iodination was from Amersham (Amersham, Bucks., U.K.) and cyclic 2'-O-succinyl-AMP 3'-[125]iodotyrosine methyl ester was from DuPont (U.K.) Ltd. (Stevenage, Herts., U.K.). The antiseraum to cyclic AMP was generously given by Dr. D. Sugden (King’s College London). Rp-adenosine 3',5'-cyclic phosphorothioate (Rp-cAMPS) was obtained from BioLog Life Science Institute (Bremen, Germany). All other reagents were of analytical grade from BDH (Poole, Dorset, U.K.). Rats (Wistar WAG; 150–200 g) were supplied by Charing Cross Hospital Medical School (London, U.K.).

Islet isolation and insulin secretion

Islets of Langerhans were isolated from fed Wistar rats by collagenase digestion of the exocrine pancreas (Bjaaland et al., 1988). Groups of three islets were incubated at 37 °C in 600 $\mu$l of a bicarbonate-buffered (pH 7.4) physiological salt solution (Gey and Gey, 1936) supplemented with 2 mM glucose, 2 mM CaCl$_2$, 0.5 mg/ml BSA and test substances of interest. Ascorbic acid (100 $\mu$M) was included to prevent oxidation of NA. In some experiments, islets were preincubated with Rp-cAMPS (500 $\mu$M) at room temperature for 1 h before addition of test substances and a further incubation for 1 h at 37 °C. Insulin content of the supernatant was determined by radioimmunoassay (Jones et al., 1988). For studies of the dynamic regulation of insulin secretion, groups of 100 islets were loaded on to nylon filters (1 $\mu$m pore size).

Abbreviations used: NA, noradrenaline; PKC, protein kinase C; PMA, phorbol myristate acetate; db cAMP, dibutyryl cyclic AMP; Rp-cAMPS, Rp-adenosine 3',5'-cyclic phosphorothioate; DAG, diacylglycerol; IBMX, 3-isobutyl-1-methylxanthine.

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perifused (37 °C, 1 ml/min) with bicarbonate buffer supplemented with agents of interest. Fractions were collected at 2 min intervals and analysed for insulin content by radioimmunoassay.

PKC down-regulation

In some studies, islet PKC was depleted by prolonged (23 h) exposure to 4βPMA, as previously described (Persaud et al., 1989b). Briefly, islets were isolated under aseptic conditions and maintained in RPMI 1640 medium supplemented with 11 mM glucose, 100 μg/ml streptomycin, 100 units/ml penicillin, 10% (v/v) foetal-calf serum and 200 nM 4βPMA or the inactive analogue, 4αPMA, in a humidified atmosphere of air/CO₂ (19:1). Islets were harvested before use, and insulin secretion was measured in static incubations, as described above.

Cyclic AMP generation

Groups of 15 freshly isolated islets were incubated for 15 min at 37 °C in bicarbonate-buffered salt solution in the presence of 1 mM IBMX and test substances of interest. The islets were pelleted by brief centrifugation (9000 g, 15 s), the supernatant was removed for assay of insulin content, and 400 μl of sodium acetate (50 mM, pH 6.2) was added to the islet pellet. Samples were boiled and sonicated (4 × 15 s, 10 μm amplitude) before assay of cyclic AMP content (Harper and Brooker, 1975).

Statistical analysis

All results are expressed as means ± S.E.M. Differences between means were analysed by Student’s unpaired t tests and considered significant when P < 0.05.

RESULTS

Time course of NA inhibition of insulin secretion

Figure 1 shows the effects of NA on the biphasic secretory response of perifused islets to 20 mM glucose. The inhibition of insulin secretion by NA was rapid in onset, such that in islets perifused in the presence of both 20 mM glucose and 10 μM NA there was a complete suppression of both first and second phases of the secretory response.

Effect of PKC down-regulation on NA inhibition of insulin secretion

After islets had been cultured in the presence of 200 nM 4βPMA for 23 h, an experimental protocol which depletes islet PKC activity (Persaud et al., 1989b), they responded significantly to 20 mM glucose, and NA completely abolished this secretory response, as it did in the control 4αPMA-treated islets (Table 1). As expected, PKC-depleted islets did not respond to a subsequent exposure to 4βPMA.

Effect of 4βPMA on NA inhibition of insulin secretion

4βPMA (150 nM) significantly potentiated the insulin-secretory response to 20 mM glucose (Figure 2). All four concentrations of NA used (1.25, 2.5, 5 and 10 μM) completely inhibited glucose-induced insulin release, but did not abolish secretion stimulated by 150 nM 4βPMA and 20 mM glucose. Even 10 μM NA, a concentration which completely suppressed secretion in response to 20 mM glucose alone (also see Figure 1), only partially inhibited the secretory response in the presence of 150 nM 4βPMA. Increasing 4βPMA to 500 nM, a maximal

![Figure 1](https://example.com/figure1.png)

**Figure 1** Time course of NA inhibition of glucose-stimulated insulin secretion

Groups of 100 islets were initially perifused with a medium containing 2 mM glucose, then (arrow) with 20 mM glucose in the absence (○) or presence (●) of 10 μM NA. Both the first short phase of secretion (10–18 min) and the prolonged second phase (20 min onwards) were abolished by NA. Points show mean of two separate experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin secretion (ng/h per islet)</th>
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<tbody>
<tr>
<td></td>
<td>4αPMA-treated</td>
</tr>
<tr>
<td>2 mM glucose</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>20 mM glucose</td>
<td>1.76 ± 0.24</td>
</tr>
<tr>
<td>20 mM glucose + 500 nM 4βPMA</td>
<td>6.13 ± 0.71</td>
</tr>
<tr>
<td>20 mM glucose + 10 μM NA</td>
<td>0.14 ± 0.01</td>
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</table>

![Figure 2](https://example.com/figure2.png)

**Figure 2** Effect of 4βPMA on NA inhibition of insulin secretion

Glucose-induced insulin release (●) from intact islets was significantly (P < 0.005) potentiated by 150 nM 4βPMA (■) and completely inhibited by NA (1.25–10 μM). All concentrations of NA significantly (P < 0.005) inhibited secretion stimulated by 4βPMA in the presence of 20 mM glucose, but there was still a significant (P < 0.005) stimulation of secretion above basal rates (2 mM glucose; △). Values are means ± S.E.M. (n = 4–5).
Figure 3. Effect of 4βPMA on NA inhibition of insulin secretion in the presence or absence of exogenous glucose

(a) 4βPMA at a maximal stimulatory concentration (500 nM) significantly (P < 0.001) potentiated glucose-stimulated insulin secretion. In the presence of 4βPMA, NA (10 μM) significantly (P < 0.001) inhibited secretion, but not to basal unstimulated levels (i.e. no glucose). Bars show means ± S.E.M. (n = 9). (b) 4βPMA (125–500 nM) significantly (P < 0.05) stimulated insulin release in the absence of added glucose (○), and at all doses of 4βPMA this increase was abolished by NA (10 μM; □). Bars show means ± S.E.M., n = 7–8.

stimulatory concentration, did not further relieve the inhibition by 10 μM NA (Figure 3a). The data shown in Figure 2 might suggest two components of the NA inhibitory effect; it is possible that NA is inhibiting secretion in response to glucose alone, while having no effect on the incremental increase due to 4βPMA. However, this appears not to be the case, because NA can also inhibit insulin release initiated by 4βPMA (125–500 nM) in the absence of exogenous glucose (Figure 3b).

Combined effect of db cAMP and 4βPMA on NA inhibition of insulin release

As db cAMP (Jones et al., 1987) and 4βPMA (Figures 2 and 3) partially relieve NA inhibition of 20 mM glucose-stimulated insulin secretion independently, the effects on secretion of maximal stimulatory concentrations of both agents, in the absence and presence of NA, were investigated. Table 2 shows that individually db cAMP (5 mM) and 4βPMA (500 nM) significantly potentiated insulin release at 20 mM glucose. Secretion in the presence of both db cAMP and 4βPMA was again significantly elevated above levels obtained with 20 mM glucose alone, and the effects of the two agents appeared to be additive. In the presence of both db cAMP and 4βPMA, NA (10 μM) significantly inhibited insulin release, but it did not fully suppress secretion to basal levels as it did in the presence of 20 mM glucose alone (see Figures 1–3).

Table 2. Effect of 4βPMA and db cAMP on NA inhibition of insulin secretion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin secretion (% of 20 mM glucose-induced)</th>
</tr>
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<tbody>
<tr>
<td>2 mM glucose</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>20 mM glucose</td>
<td>100 ± 14</td>
</tr>
<tr>
<td>20 mM glucose + 5 mM db cAMP</td>
<td>179 ± 30</td>
</tr>
<tr>
<td>20 mM glucose + 500 nM 4βPMA</td>
<td>297 ± 39</td>
</tr>
<tr>
<td>20 mM glucose + 5 mM db cAMP + 500 nM 4βPMA</td>
<td>404 ± 38</td>
</tr>
<tr>
<td>+ 500 nM 4βPMA</td>
<td>143 ± 22</td>
</tr>
</tbody>
</table>

Table 3. Effect of 4βPMA on NA inhibition of insulin secretion and cyclic AMP accumulation

Groups of 15 freshly isolated islets were incubated for 15 min in the presence of test agents of interest and 1 mM IBMX, after which time insulin content of the supernatant and cyclic AMP content of the islet pellet were assessed by radioimmunoassay. At 20 mM, glucose stimulated a 2-fold increase in cyclic AMP generation (P < 0.001), and this was significantly (P < 0.001) inhibited by NA (10 μM). 4βPMA (500 nM) did not significantly increase cyclic AMP (P > 0.2 versus 20 mM glucose), but prevented NA from completely inhibiting cyclic AMP generation (P < 0.001 versus 2 mM glucose), concomitant with a significant alleviation of the inhibitory effect of NA on insulin release (P < 0.001 versus 20 mM glucose + 10 μM NA). Values are means ± S.E.M. (n = 9–10).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cyclic AMP (fmol/15 min per islet)</th>
<th>Insulin (ng/15 min per islet)</th>
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<tr>
<td>2 mM glucose</td>
<td>24.7 ± 1.7</td>
<td>0.43 ± 0.05</td>
</tr>
<tr>
<td>20 mM glucose</td>
<td>54.1 ± 4.8</td>
<td>1.02 ± 0.04</td>
</tr>
<tr>
<td>20 mM glucose + 10 μM NA</td>
<td>28.2 ± 2.2</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>20 mM glucose + 500 nM 4βPMA</td>
<td>65.6 ± 7.7</td>
<td>1.90 ± 0.11</td>
</tr>
<tr>
<td>+ 500 nM 4βPMA</td>
<td>42.7 ± 3.0</td>
<td>0.81 ± 0.05</td>
</tr>
</tbody>
</table>

Effect of 4βPMA on cyclic AMP production and insulin release in the absence and presence of NA

The inability of a combination of maximal secretory concentrations of 4βPMA and db cAMP to elicit a full secretory response in the presence of 10 μM NA suggests that NA has inhibitory effects distal to cyclic AMP and DAG generation and that the partial alleviation of inhibition by each agent might be by the same intracellular pathway. Table 3 shows insulin release and cyclic AMP accumulation measured in the same islets after a 15 min incubation period at 37 °C. The effects of 4βPMA on
secretion in the presence and absence of 10 μM NA are similar to those presented in Figures 2 and 3(a). At 20 mM glucose, stimulated a 2-fold increase in cyclic AMP production, and this was significantly inhibited by NA. 4βPMA (500 nM) significantly potentiated secretion, and had a small but insignificant stimulatory effect on cyclic AMP accumulation at 20 mM glucose. In the presence of 4βPMA, the inhibitory effects of NA on both insulin release and cyclic AMP generation were significantly decreased.

**Effect of Rp-cAMPs on the alleviation of NA inhibition of insulin secretion by PMA**

Figure 4 shows the effect of the competitive antagonist of cyclic AMP-dependent protein kinase, Rp-cAMPs (50 μM), on the ability of 4βPMA to relieve NA inhibition of insulin secretion. In the presence of 500 nM 4βPMA, NA did not exert a full inhibition of glucose-stimulated insulin release, whether or not islets had been preincubated for 1 h with P-AMPs. As expected, Rp-cAMPs significantly inhibited the potentiation of insulin secretion by the adenylate cyclase activator forskolin, but had no effect on insulin secretion in response to 20 mM glucose alone. Somewhat surprisingly, the stimulatory effect of 4βPMA at 20 mM glucose was decreased in islets which had been exposed to Rp-cAMPs for 1 h.

**DISCUSSION**

The well-documented inhibitory effect of NA on glucose-stimulated insulin secretion (reviewed by Morgan, 1987) is confirmed by the present studies. Our earlier studies, and those of other groups, have indicated that NA inhibits insulin secretion by interacting with at least one pertussis-toxin-sensitive GTP-binding protein, and full inhibition of the secretory response is not obtained solely by decreasing the intracellular levels of cyclic AMP and Ca²⁺ (Ullrich and Wolhime, 1988; Persaud et al., 1989a). The other signalling systems which mediate the actions of NA within pancreatic B-cells have not been identified, but in other cell types activators of PKC are known to modify the cellular responses to adrenergic agonists (Johnson et al., 1986).

Our experiments with PKC-depleted islets demonstrate that activation of PKC is not required for the full expression of adrenergic inhibition of glucose-stimulated insulin secretion. However, activation of PKC with 4βPMA partially alleviated NA inhibition of glucose-induced insulin release. Our previous studies have indicated that the inhibitory capacity of NA was also decreased, but not abolished, in the presence of cyclic AMP, and this has been attributed to maintenance of cyclic AMP levels despite the inhibition of adenylate cyclase activity by NA (Jones et al., 1987). It is unlikely that 4βPMA decreases NA inhibition of secretion in an analogous manner, by maintaining levels of DAG-like material distal to inhibition of phospholipase C activity, because catecholamines do not inhibit phosphoinositide hydrolysis in islets (Axen et al., 1983; Montague et al., 1985; Blachier et al., 1987).

One possible mechanism of action of 4βPMA could be a blockade of NA inhibition of adenylate cyclase activity. In human platelets and c-lymphoma cells (Jakobs et al., 1985; Katada et al., 1985) 4βPMA has been shown to impair specifically inhibition of adenylate cyclase activity by agonists acting via Gi, an effect mediated by phosphorylation of a 41 kDa protein, identified as bGI. In the present studies, 4βPMA impaired the inhibitory effect of NA on islet cyclic AMP generation. Similarly, it has been reported that somatostatin inhibition of islet adenylate cyclase activity is decreased in the presence of 1.6 μM 4βPMA (Thams et al., 1988). It is possible that in islets activation of PKC results in phosphorylation of the α subunit of the inhibitory G-protein linked to adenylate cyclase, relieving the suppression of cyclic AMP generation. In this way, 4βPMA would prevent NA inhibiting secretion to basal levels in the same way as does db cAMP, i.e. by bypassing the inhibition of adenylate cyclase. In support of this model is the observation that 4βPMA enhances phosphorylation of a 40 kDa protein in both intact (Harrison et al., 1984) and electrically permeabilized (Jones et al., 1988) islets of Langerhans, but this protein has not been positively identified as GIₐ.

However, our experiments in which Rp-cAMPs, a membrane-permeant competitive antagonist of protein kinase A, did not prevent 4βPMA alleviation of NA inhibition of insulin secretion point to a different mode of action of 4βPMA. We have previously reported that preincubation of islets with Rp-cAMPs inhibits cyclic AMP-induced protein phosphorylation and insulin secretion stimulated by cyclic AMP or forskolin (Persaud et al., 1990). Thus effects on the secretory process which are mediated by elevations in cyclic AMP should be inhibited by Rp-cAMPs. Our present experiments demonstrate that, when the second-messenger effects of cyclic AMP were prevented by treatment of islets with Rp-cAMPs, 4βPMA still alleviated the inhibitory effects of NA on insulin secretion. It is noteworthy that, as with inhibiting forskolin potentiation of insulin secretion, Rp-cAMPs decreased the potentiating capacity of 4βPMA. Although 4βPMA did not significantly stimulate cyclic AMP levels in our experiments, several groups have reported increased islet cyclic AMP production by 4βPMA (Malaisse et al., 1980; Bozem et al., 1987; Thams et al., 1988). A possible explanation for the lack of effect of 4βPMA on cyclic AMP concentrations in the experiments shown here is the short incubation time (15 min); Malaisse et al. (1980) have reported that 4βPMA only enhances cyclic AMP production after a 60 min incubation period. It is therefore possible that activation of PKC by 4βPMA results in potentiation of insulin release in part by the generation of cyclic AMP.
The present studies suggest that the activation of PKC has at least two distinct effects on the B-cell secretory mechanism. Thus, in the absence of glucose, the insulin-secretory response to 4β-PMA, like that to stimulatory concentrations of glucose, is totally inhibited by NA. However, in the presence of glucose, 4β-PMA provides protection against the full inhibitory effects of NA which are observed in the presence of either agent individually. The simplest explanation for this is that activation of PKC may be alleviating inhibition of insulin secretion by maintaining levels of a second messenger generated in response to glucose which is normally inhibited by NA. The most obvious candidate, cyclic AMP, has been eliminated by the present experiments, and, in any case, cyclic AMP is not required for glucose-stimulated insulin release (Persaud et al., 1990). Other second messengers which are increased by glucose, such as Ca²⁺ (Wollheim and Sharp, 1981) or arachidonic acid (Wolf et al., 1986), must be considered, but further experiments are required to determine whether inhibition of their generation by NA can be relieved by the activation of PKC.

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REFERENCES


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