ELSEVIER

European Journal of Pharmacology 324 (1997) 227–232

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The effect of selective phosphodiesterase inhibitors on plasma insulin concentrations and insulin secretion in vitro in the rat

Mona El-Metwally [1], Reza Shafiee-Nick, Nigel J. Pyne, Brian L. Furman *

Department of Physiology and Pharmacology, University of Strathclyde, George Street, Glasgow G1 1XW, UK

Received 20 January 1997; accepted 24 January 1997

Abstract

We have examined in rats the effects of Org 9935 (4,5-dihydro-6-(5,6-dimethoxy-benzo[*b*]-thien-2-yl)-methyl-1-(2*H*)-pyridazinone), a selective inhibitor of type 3 phosphodiesterase (phosphodiesterase 3) and Org 30029 (*N*-hydroxy-5,6-dimethoxy-benzo[*b*]-thiophene-2-carboximidamide HC1), an inhibitor of phosphodiesterase 3/4 on rat plasma insulin and glucose concentrations in pentobarbitone-anaesthetised rats and on insulin secretion by rat isolated islets. We have also compared their effects on islet phosphodiesterase activity. Org 9935 (0.1 and 1.0 mg kg^{-1} i.v. 15 min previously) dose dependently elevated fasting and post-glucose (0.25 g kg^{-1} i.v.) plasma insulin concentrations. Org 30029 in a dose of 10 mg kg^{-1}, but not 1 mg kg^{-1}, also increased plasma insulin concentrations. Neither drug modified either fasting or post-glucose plasma glucose concentrations. Each drug augmented glucose-induced insulin release by rat isolated islets in a static incubation system, with approximate EC_{50} values of 1.5 μM for Org 9935 and 20 μM for Org 30029. Phosphodiesterase activity, in both supernatant and pellet fractions of islet homogenates, was inhibited concentration dependently by each drug. Although the shape of the concentration-inhibition curve for Org 30029 precluded estimation of an IC_{50} value, this drug was clearly much less potent than Org 9935 (IC_{50} about 50 nM) in inhibiting islet phosphodiesterase activity. We conclude that the increase in plasma insulin produced by each drug is a consequence of augmented insulin secretion, probably secondary to inhibition of phosphodiesterase 3 in the islet β cell, with a resultant elevation in cAMP. The failure of the drugs to modify plasma glucose may be due to concomitant inhibition of cAMP phosphodiesterase in liver and adipose tissue.

Keywords: Org 9935; Org 30029; Insulin secretion; Phosphodiesterase inhibitor; Insulin, plasma; Glucose, plasma

1. Introduction

Cyclic 3′5′-AMP (cAMP) is probably an important intracellular modulator of glucose-induced insulin secretion (Zawalich and Rasmussen, 1990) and mediates the insulinotropic action of the major incretin factor glucagon-like peptide 1 (GLP-1) (Drucker et al., 1987). Cyclic nucleotide phosphodiesterases terminate the actions of cAMP by promoting its degradation to 5′-AMP. Seven main phosphodiesterase isoenzymes have been identified, which show different substrate specificities and different susceptibility to inhibition by drugs (Beavo et al., 1994). Moreover, these isoenzymes show different distributions and functional importance among various tissues and this may be exploited in the drug treatment of various diseases.

We and others have recently shown that phosphodiesterase 3 (cGMP-inhibited, high affinity for both cAMP and cGMP) is present in rat and human islets (Furman and Pyne, 1990; Shafiee-Nick et al., 1994, 1995; Parker et al., 1995). This isoform appears to be functionally important, since relatively selective phosphodiesterase 3 inhibitors Org 9935, siguazodan), but not zaprinast (phosphodiesterase 1/5 inhibitor) or rolipram (phosphodiesterase 4 inhibitor) augmented glucose-induced insulin secretion from isolated islets. There is no information about the in vivo effects of phosphodiesterase 3 inhibitors on insulin secretion or blood glucose. Therefore the present work was undertaken to examine the effects of Org 9935, a specific phosphodiesterase 3 inhibitor and Org 30029, a mixed phosphodiesterase 3/4 inhibitor (Shahid et al., 1991) on plasma glucose and immunoreactive insulin concentrations in the rat. A comparison was also made of their in vitro potencies in inhibiting rat islet phosphodiesterase and in augmenting glucose-induced insulin secretion from rat isolated islets.

* Corresponding author.

[1] Present address: Department of Pharmacology, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

0014-2999/97/$17.00
PII S0014-2999(97)00076-9

2. Materials and methods

The following drugs and chemicals were used: pentobarbitone sodium (Sagatal, W.J. Dunlop); Org 9935 (4,5-dihydro-6-(5,6-dimethoxy-benzo[*b*]-thien-2-yl)-methyl-1(2H)-pyridazinone) and Org 30029 (*N*-hydroxy-5,6-dimethoxy-benzo[*b*]-thiophene-2-carboximidamide HCl), generous gifts from Organon Laboratories, Newhouse, UK); [^{3}H]cyclic AMP (Amersham International, Amersham, UK); dimethyl sulphoxide (Sigma, Poole, UK). Org 9935 and Org 30029 were dissolved in dimethyl sulfoxide (DMSO) and an appropriate volume of DMSO was used as control vehicle.

2.1. In vivo studies

Male Sprague-Dawley rats (250–300 g) were allowed free access to water until the experiment and were either fasted for 18 h or allowed free access to their normal diet (CRM; supplied by BS&S, Edinburgh, UK) as specified. All experiments were performed in compliance with the Animals (Scientific Procedures) Act (1986). The animals were anaesthetised with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and allowed to breathe spontaneously. The left common carotid artery and right femoral vein were cannulated for blood sampling/blood pressure recording and drug administration respectively. Blood pressure was recorded using a Statham pressure transducer coupled to a chart recorder. Body temperature was maintained at 37 ± 0.5°C using a rectal thermistor probe coupled to a lamp positioned over the abdomen.

2.2. Isolated islets

Islets were prepared according to the method of Lacy and Kostianovsky (1967) and insulin secretion examined as described previously using batches of 5 islets in a static incubation system (Shafiee-Nick et al., 1995). Previous work in this laboratory has validated the system for studying isolated islets and has shown, for example, insulin release to be inhibited by calcium channel blocking agents and by α_2-adrenoceptor blocking drugs (Semple et al., 1988; Smith and Furman, 1988).

2.3. Biochemical determinations

Plasma glucose was determined using a glucose analyser (Beckman GLU2, Beckman Instruments, Glenrothes, Fife). Immunoreactive insulin in plasma or incubation medium was determined using a commercial kit (ICN Biomedicals, Thame, Oxfordshire, UK) and a pure, crystalline, rat insulin standard (Novo-Nordisk, Copenhagen, Denmark).

The effect of drugs on islet phosphodiesterase activity was determined in batches of 100 islets as reported previously (Shafiee-Nick et al., 1995) using the method of Thompson and Appleman (1971). Activity was expressed as pmol cyclic AMP ml^{-1} min^{-1} or as a percentage of control.

2.4. Expression of results and statistical analysis

Glucose data (mM), blood pressure data (mmHg) or in vitro insulin secretion data (ng islet^{-1} h^{-1}) were expressed as means ± S.E.M. Plasma immunoreactive insulin concentrations (ng ml^{-1}) were expressed as geometric means with 95% confidence intervals. Statistical significance was determined using Student's *t*-test for unpaired comparisons or analysis of variance (ANOVA) followed by Student's *t*-test, using the Bonferroni correction for multiple comparisons. Statistical analysis of plasma immunoreactive insulin data was performed after log transformation.

Table 1
Plasma concentrations of immunoreactive insulin (ng ml^{-1}, geometric means, 95% confidence limits) at various times after injection of glucose (0.25 g kg^{-1} i.v.) 15 min after either drug or control solutions in fasted rats

Treatment	Plasma insulin (ng ml^{-1})				
	Zero time [d]	5 min	10 min	15 min	20 min
Control	1.5 (1.2, 2.0)	3.1 (1.6, 6.1)	3.9 (2.3, 6.6)	2.3 (1.1, 4.8)	3.5 (1.9, 6.7)
Org 9935 (0.1 mg kg^{-1})	4.8 [a] (2.6, 8.6)	11.4 [b] (7.5, 17.1)	11.4 [a] (6.9, 18.8)	8.2 [a] (5.0, 13.6)	6.1 (2.8, 13.4)
Org 9935 (1.0 mg kg^{-1})	7.6 [b] (4.9, 11.8)	15.2 [b] (7.9, 29)	21.8 [c] (13.3, 36)	18.1 [c] (13.4, 24.4)	14.4 [a] (11, 19.0)
Org 30029 (1 mg kg^{-1})	2.5 (1.3, 4.7)	7.5 (4.3, 12.8)	6.1 (2.7, 13.9)	5.5 (2.4, 12.6)	5.4 (2.7, 10.7)
Org 30029 (10 mg kg^{-1})	5.1 [a] (2.4, 10.8)	10.0 [a] (5.8, 17.4)	15.6 [c] (9.2, 26.2)	16.0 [c] (9.8, 26.2)	12.7 [a] (6.5, 24.4)

Each value is the geometric mean of 6 observations with 95% confidence limits. Bonferroni P value vs. control group (after ANOVA).
[a] ($P < 0.05$); [b] ($P < 0.01$); [c] ($P < 0.001$).
[d] Measured just before glucose injection.

3. Results

3.1. Plasma immunoreactive and plasma glucose concentrations

Org 9935 produced an increase in fasting and glucose-elevated plasma immunoreactive concentrations, which appeared to be dose-dependent (Table 1). Only the larger dose of Org 30029 increased insulin concentrations significantly (Table 1). There was no significant effect of either drug on plasma glucose concentrations (Table 2).

3.2. Insulin secretion in vitro

Org 9935 and Org 30029 each produced a concentration-dependent increase in insulin release in response to glucose (10 mM, Fig. 1), without significantly modifying basal insulin secretion in 3 mM glucose from the control value of 1.8 ± 2 ng islet^{-1} h^{-1}. Org 9935 (EC_{50} 1.5 μM) was about 10-fold more potent than Org 30029 (EC_{50} 20 μM).

3.3. Islet phosphodiesterase activity

Org 30029 produced a concentration-dependent inhibition of islet phosphodiesterase activity, which was similar in both pellet and supernatant fractions (Fig. 2). The shape of the inhibition curves did not allow calculation of IC_{50} values but, assuming that the highest concentration tested was producing maximum inhibition, the IC_{50} would be in the range 50–100 μM. Org 9935 also produced a concentration-dependent inhibition of islet phosphodiesterase activity with an IC_{50} in the pellet fraction of about 50 nM (Fig. 2).

3.4. Blood pressure and heart rate

Blood pressure (119 ± 10 mmHg) and heart rate (409 ± 15 beats/min) in control rats was stable after saline throughout the 15 min study period. Org 9935 and Org 30029 each produced an immediate fall in mean arterial blood pressure. Mean arterial blood pressure values (means ± S.E.M.) at 1 min after injection were Org 9935, 0.1 mg

Table 2
Plasma concentrations of glucose (mmol l^{-1}, mean ± S.E.M.) at various times after injection of glucose (0.25 g kg^{-1} i.v.) 15 min after either drug or control solutions in fasted rats

Treatment	Plasma insulin (ng ml^{-1})				
	Fasting [a]	5 min	10 min	15 min	20 min
Control	6.4 ± 0.1	12.5 ± 0.2	10.9 ± 0.3	9.4 ± 0.4	8.2 ± 0.2
Org 9935 (0.1 mg kg^{-1})	6.5 ± 0.2	13.3 ± 0.5	10.8 ± 0.5	8.7 ± 0.5	7.3 ± 0.5
Org 9935 (1.0 mg kg^{-1})	5.9 ± 0.2	12.2 ± 1.0	9.7 ± 0.9	7.2 ± 0.8	5.5 ± 0.7
Org 30029 (1 mg kg^{-1})	6.9 ± 0.41	14.9 ± 1.0	13.3 ± 0.7	12.0 ± 0.8	10.8 ± 0.9
Org 30029 (10 mg kg^{-1})	7.0 ± 0.4	15.5 ± 1.0	13.1 ± 0.9	11.7 ± 0.9	10.2 ± 1.2

Each value is the mean ± S.E.M. of 6 observations.
[a] Measured just before glucose injection.

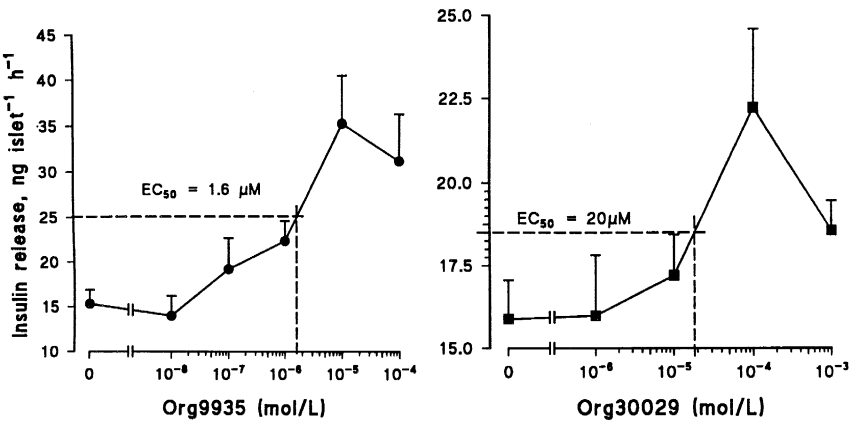

Fig. 1. Effect of different concentrations of Org 9935 (left panel) and Org 30029 (right panel) on insulin secretion from isolated islets in response to 10 mM glucose. Batches of 5 islets were incubated for 60 min. Insulin secretion in 3 mM glucose without drug was 1.8 ± 0.2 ng islet^{-1} h^{-1}. Each point is the mean ± S.E.M. of 9–12 observations. The EC_{50} values are derived from the mean dose-response curves and are the concentrations producing 50% of the maximum responses.

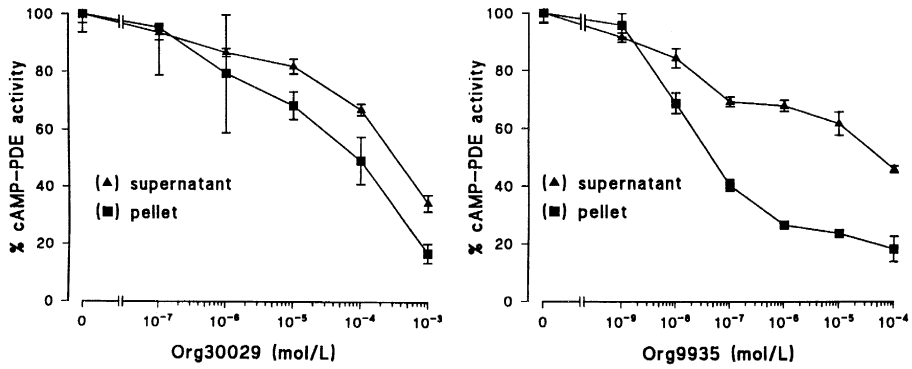

Fig. 2. Effect of different concentrations of Org 30029 (left panel) and Org 9935 (right panel) on cAMP phosphodiesterase activity in pellet and supernatant fractions of homogenates of rat islets. The absolute values in the absence of drugs, were, respectively, for the pellet and supernatant fractions 31.5 ± 6.2 and 46.2 ± 10 pmol min^{-1} ml^{-1} for experiments using Org 9935 and 36.5 ± 6.5 and 57.3 ± 10.6 for the experiments using Org 30029. The left panel is modified from Shafiee-Nick et al. (1995) with the permission of the publisher. Each value is the mean ± S.E.M. of 3 separate experiments.

kg^{-1}, 47 ± 2.6 mmHg ($P < 0.05$), 1 mg kg^{-1}, 43.5 ± 4 mmHg ($P < 0.001$), Org 30029 1 mg kg^{-1}, 48 ± 5 mmHg, 10 mg kg^{-1}, 51 ± 9 mmHg ($P < 0.01$). The hypotensive response to Org 9935 at each dose persisted for at least 15 min, whereas blood pressure had returned to control levels by 5 min after administration of Org 30029. Neither drug had any significant effect on heart rate.

4. Discussion

We have previously reported that selective inhibitors of phosphodiesterase 3 augment glucose-induced insulin secretion from rat islets in vitro (Shafiee-Nick et al., 1995). Agents that elevate islet β cell cAMP levels, such as forskolin or GLP-1, were also shown to increase circulating immunoreactive insulin concentrations (Ammon and Muller, 1984; Dalessio et al., 1994). Plasma insulin concentrations are also increased by non-selective, methylxanthine phosphodiesterase inhibitors (Giugliano et al., 1988) but the effects of selective phosphodiesterase inhibitors have not been investigated in vivo.

The increase in plasma immunoreactive insulin concentrations following administration of either Org 9935 or Org 30029 is in accord with their direct effects on insulin release from isolated islets, which we have also reported previously for Org 9935 (Shafiee-Nick et al., 1995). The drugs increased insulin secretion in vitro only in the presence of a stimulatory glucose concentration but without any effect on basal insulin secretion. This has been reported previously for drugs that increase cyclic 3′5′AMP within the islet β cell. Thus, in rat isolated islets, forskolin had relatively little effect on basal insulin secretion but had a much more marked effect at threshold or stimulatory glucose concentrations (Henquin and Meissner, 1984a). Similarly, GLP-1 was more effective in enhancing insulin secretion in vitro at stimulatory glucose concentrations (Hargrove et al., 1996). A similar glucose dependency was observed for the cAMP analogue dibutyryl cAMP which augmented glucose-induced, but not basal, insulin secretion by rat islets (Henquin and Meissner, 1984b). From these observations one may have anticipated that Org 9935 and Org 30029 might be relatively ineffective in elevating circulating insulin concentrations in fasted rats, before the injection of glucose. However, although the rats used in the present work for the in vivo experiments were fasted for 18 h, the plasma glucose concentration in controls was 6.4 ± 0.1 mmol l^{-1}, which is around the threshold for stimulation of insulin secretion. Org 9935 was clearly more potent than Org 30029, with 0.1 mg kg^{-1} producing marked hyperinsulinaemia. Org 30029 was ineffective at a dose of 1 mg kg^{-1}, hyperinsulinaemia being seen only at a dose of 10 mg kg^{-1}. The relative in vivo potency of these compounds may also be influenced by pharmacokinetic factors.

As reported previously for Org 9935 (Shafiee-Nick et al., 1995) Org 30029 inhibited islet phosphodiesterase activity in a concentration-dependent manner. However, the shape of the curve precluded any calculation of an IC_{50} concentration. While Org 9935 appears to be selective for phosphodiesterase 3, Org 30029 inhibits both phosphodiesterase 3 and phosphodiesterase 4 (Shahid et al., 1991). This may explain the inability to show a maximal effect at less than 100% inhibition, as phosphodiesterase 4 may be present in islets, as evidenced by some inhibitory effects of rolipram and ICI 63197 on islet phosphodiesterase activity (Furman and Pyne, 1990: Shafiee-Nick et al., 1995). However, as the IC_{50} for Org 9935 was estimated to be 50 nM, Org 30029 is clearly less potent, with inhibition not clearly evident at less than 1 μM. The relative potencies of the two drugs in inhibiting islet phosphodiesterase activity is in accord with their relative potencies in augmenting insulin secretion in vitro and in increasing plasma immunoreactive insulin concentrations. In absolute terms, Org 9935 is much more potent in inhibiting islet phosphodiesterase activity than in increasing insulin secretion from isolated islets. This may reflect the use of cell homogenates for the phosphodiesterase assay compared with

intact islets for the insulin secretion studies and may relate to the permeability of the islet to drug. In contrast, Org 30029 appears to be roughly equipotent in inhibiting phosphodiesterase activity and in enhancing insulin release in vitro. The role of cAMP in mediating the effects of these compounds must remain speculative. Org 9935 and siguazodan, another selective phosphodiesterase 3 inhibitor, increases islet cAMP only when adenylyl cyclase is activated using forskolin (Shafiee-Nick et al., 1995). This is probably due to the action of other phosphodiesterases that are not inhibited by these drugs.

Despite markedly elevating circulating insulin concentrations, neither drug modified either fasting, or post-glucose, plasma glucose concentrations. Forskolin, an activator of adenylyl cyclase also increases the plasma insulin response to glucose, without modifying plasma glucose concentrations (Ammon and Muller, 1984). Increases in cAMP produced by forskolin or by phosphodiesterase inhibitors in adipose tissue or liver may prevent the effects of insulin on these tissues. Phosphodiesterase 3 appears to be functionally important in both adipose tissue and liver and its activation by insulin in these tissues contributes to the effects of this hormone (Heyworth et al., 1983; Pyne et al., 1987; Hagstromtoft et al., 1995; Manganiello et al., 1995). Methylxanthine, non-selective phosphodiesterase inhibitors were reported to produce hyperglycaemia, in part through activation of the sympathetic nervous system (Vestal et al., 1983; Leblanc et al., 1995). In this context, it is noteworthy that in doses that increased plasma insulin concentrations Org 9935 and Org 30029 each produced a marked drop in blood pressure, possibly as a consequence of inhibition of phosphodiesterase 3 in vascular smooth muscle. This may result in reflex activation of the sympathetic nervous system, although the absence of tachycardia may argue against this possibility.

In conclusion, Org 9935, a selective inhibitor of phosphodiesterase 3, and Org 30029, an inhibitor of phosphodiesterase 3/4, each augmented glucose-induced insulin secretion in vitro and increased plasma immunorective insulin concentrations. These effects may relate to inhibition of islet phosphodiesterase activity and further suggest the functional importance of phosphodiesterase 3 in the pancreatic islet β cell. The inability of the selective phosphodiesterase inhibitors to produce hypoglycaemia suggests that inhibitors of β cell phosphodiesterase are unlikely to yield useful oral hypoglycaemic agents. However, a recent, novel hypoglycaemic agent, apparently working via stimulation of insulin secretion, was shown to be a phosphodiesterase inhibitor (Leibowitz et al., 1995). Either this drug is fortuitously selective for the β cell phosphodiesterase 3, or it has additional properties conferring hypoglycaemic activity. Phosphodiesterase 3 is now known to exist as two isoforms which show different tissue distribution (Miki et al., 1996) and which may differ in their sensitivities to drugs, although this is, as yet, unknown.

Acknowledgements

We are grateful to Organon Laboratories Newhouse, Lanarkshire, Scotland, UK for generous supplies of Org 9935 and Org 30029, to the British Council and the University of Alexandria for supporting Dr El-Metwally and to the Iranian Ministry of Health for a grant to Dr Shafiee-Nick.

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