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Does cyclic guanosine monophosphate mediate noradrenaline-induced inhibition of islet insulin secretion stimulated by glucose and palmitate?

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Noradrenaline inhibits in rat islets the stimulation of insulin secretion induced by glucose and its potentiation by palmitate, but the signalling system responsible remains unknown. We have tested the hypothesis that noradrenaline-induced inhibition is mediated by an elevation of cyclic GMP (cGMP) levels. The analogue 8-Br-cGMP decreases dose-dependently the potentiation by palmitate of glucose-induced insulin secretion, whereas it only slightly affects the proper effect of glucose. Similarly, it abolishes palmitate acceleration of glucose-induced $^{45}Ca^{2+}$ uptake without modifying the sugar effect. Finally, 8-Br-cGMP completely inhibits the stimulation of the lipid synthesis *de novo* induced by palmitate, but not that caused by glucose alone. On the other hand, noradrenaline increases dose-dependently islet cGMP content, with α_2-adrenergic specificity. As noradrenaline-induced elevation of cGMP is sensitive to pertussis toxin, it probably results from α_2-adrenoceptor activation of islet guanylate cyclase through a guanine nucleotide regulatory protein. It is concluded that the elevated cGMP levels mediate noradrenaline inhibition of lipid synthesis *de novo*, and hence of acceleration by palmitate of $^{45}Ca^{2+}$ uptake and insulin secretion in the presence of glucose.

INTRODUCTION

Palmitate accelerates dose-dependently in rat islets the glucose-stimulated rate of both insulin secretion and $^{45}Ca^{2+}$ turnover [1,2]. This acceleration of islet function is linked to the metabolism of palmitate, as it is slowed by starvation and restored by specific inhibitors of mitochondrial fatty acid oxidation [2,3]. In fact, palmitate increases dose-dependently its own esterification rate as well as that of lipid synthesis *de novo* in parallel with the potentiation of glucose-induced insulin release and $^{45}Ca^{2+}$ turnover [2,3]. Noradrenaline is a strong inhibitor of glucose-stimulated insulin secretion [4], even after its potentiation by palmitate [5]. The amine also blocks glucose-induced $^{45}Ca^{2+}$ uptake and its potentiation by palmitate with a similar sensitivity and α_2-adrenergic specificity [5]. In addition to this, both palmitate oxidation and lipid synthesis *de novo* are similarly decreased by noradrenaline, which also inhibits the glucose utilization rate [6]. This suggests that α_2-adrenoceptor stimulation may block the metabolic recognition of these nutrient stimulators of insulin release. There is also strong evidence supporting the idea that noradrenaline acts at a distal step in the stimulus–secretion coupling mechanism of β-cells [7]. However, these two hypothetical mechanisms do not exclude each other, and their simultaneous activation would reinforce α_2-adrenergic inhibition.

Whereas a guanine nucleotide regulatory protein (G-protein) seems to be directly implicated in the inhibition of a distal step in the stimulus–secretion coupling by α_2-agonists [7], the intracellular signal(s) mediating the inhibition of metabolic recognition is unknown. Glucose increases the islet content of cyclic AMP (cAMP) slightly and transiently [8], and α_2-adrenergic agents decrease it [9] according to their ability to inhibit islet adenylate cyclase [10]. However, stimulation of endogenous cAMP production by either dibutyryl cAMP [11] or forskolin [12] does not reverse α_2-adrenoceptor-mediated inhibition of glucose-induced insulin secretion.

Receptor occupation by hormones and neurotransmitters activates a G-protein, which then increases or decreases the intracellular production of a second messenger by a specific effector [13]. There is also evidence that a single occupied receptor can interact with more than one G-protein [14], leading to an altered production of several intracellular signals. It has been reported that α_2-adrenergic stimulation of adrenocortical carcinoma cells results in an elevation of cGMP and a concomitant decrease in cAMP levels [15]. Therefore, we have investigated in isolated rat islets whether noradrenaline stimulates the production of cGMP and whether exogenously added cyclic nucleotide can reproduce the amine effects on insulin secretion, lipid synthesis *de novo* and $^{45}Ca^{2+}$ uptake.

EXPERIMENTAL

Chemicals

D-[U-^{14}C]Glucose, $^{45}CaCl_2$ and Na^{125}I were from The Radiochemical Centre (Amersham, Bucks., U.K.). Activated charcoal, Hepes, standard lipids, (−)-noradrenaline bitartrate, yohimbine hydrochloride and pertussis toxin were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fatty-acid-poor BSA was from Miles Laboratories (Kankakee, IL, U.S.A.). Palmitic acid was from Serva (Heidelberg, Germany). Crystalline pig and rat insulins were kindly supplied by Novo Industri (Copenhagen, Denmark). Collagenase, 8-Br-cAMP and 8-Br-cGMP were from Boehringer (Mannheim, Germany). ^{125}I-RIA (radioimmunoassay) kits for cGMP and cAMP were from New England Nuclear (Dreieich, Germany). All organic solvents and inorganic salts were of analytical grade, from Merck (Darmstadt, Germany).

Methods

Islets were isolated by collagenase digestion [16] of the pancreas of adult Wistar Albino rats (males of 250 g body wt.). Insulin secretion was studied in batch-type incubations (three islets/ 300 μl) in Krebs–Ringer bicarbonate, buffered with 10 mM-Hepes and equilibrated with O_2/CO_2 (19:1) to pH 7.4. BSA (fatty acid

Abbreviations used: cGMP, cyclic GMP; cAMP, cyclic AMP.
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Table 1. Effect of 8-bromo cyclic nucleoside monophosphates on the insulin response to 20 mM-glucose at different palmitate concentrations

Values denote means ± S.E.M., and the number of animals used in each experimental condition is given in parentheses. Statistical comparisons were performed by the non-paired Student's *t* test. Abbreviations: 0.1cAMP, 0.25cAMP, 0.5cAMP and 1cAMP are 0.1 mM-, 0.25 mM-, 0.5 mM- and 1 mM-8-Br-cAMP respectively; 0.1cGMP, 0.25cGMP, 0.5cGMP and 1cGMP are 0.1 mM-, 0.25 mM-, 0.5 mM- and 1 mM-8-Br-cGMP respectively; 0Palm, 0.25Palm and 1Palm are 0, 0.25 mM- and 1 mM-palmitate respectively.

Nucleotides (mM)	Insulin (ng/h per islet)					
	0Palm	*P*	0.25Palm	*P*	1Palm	*P*
0	10.9 ± 0.5 (15)	Control	14.2 ± 0.6 (6)	Control	23.8 ± 0.8 (18)	Control
0.1cAMP	11.0 ± 0.4 (6)	N.S.	15.31 ± 0.8 (6)	N.S.	24.3 ± 1.2 (5)	N.S.
0.25cAMP	13.7 ± 0.6 (15)	< 0.005	18.6 ± 0.4 (6)	< 0.001	24.8 ± 1.1 (13)	N.S.
0.5cAMP	15.2 ± 0.6 (5)	< 0.001	18.9 ± 0.5 (6)	< 0.001	24.3 ± 1.4 (6)	N.S.
1cAMP	16.0 ± 0.5 (6)	< 0.001	20.31 ± 0.5 (6)	< 0.001	26.1 ± 1.0 (5)	N.S.
0.1cGMP	10.9 ± 0.3 (5)	N.S.	14.4 ± 0.5 (5)	N.S.	22.7 ± 0.8 (5)	N.S.
0.25cGMP	10.5 ± 0.4 (13)	N.S.	10.0 ± 0.6 (6)	< 0.001	16.2 ± 0.7 (15)	< 0.001
0.5cGMP	8.1 ± 0.3 (7)	< 0.005	8.7 ± 0.4 (6)	< 0.001	13.0 ± 0.7 (7)	< 0.001
1cGMP	7.9 ± 0.4 (7)	< 0.005	8.3 ± 0.6 (6)	< 0.001	10.8 ± 0.5 (6)	< 0.001
0.25cGMP + 1cAMP	13.6 ± 0.7 (13)	< 0.005	–	–	18.0 ± 1.2 (8)	< 0.001

Table 2. Effect of 1 mM-8-bromo cyclic nucleotides (cAMP and cGMP) on the rate of incorporation of 20 mM-D-[U-^{14}C]glucose into phosphatidylcholine (PC), phosphatidylethanolamine (PE), diacylglycerols (DG), triacylglycerols (TG), phosphatidic acid (PA), phosphatidylinositol (PI) and polyphosphoinositides (PPI), in the presence of 1 mM-palmitate

Values denote means ± S.E.M.; six animals were used in each experimental condition, except where indicated (*n = 13). Statistical comparisons were performed by the non-paired Student's *t* test with the corresponding controls in the absence of nucleotides.

	D-[U-^{14}C]Glucose incorporation (pmol/2h per islet)						
Nucleotide added...	None (control)	cAMP	*P*	cGMP	*P*	cAMP + cGMP	*P*
PC	2.53 ± 0.28	2.45 ± 0.16	N.S.	0.47 ± 0.07	< 0.001	0.61 ± 0.13	< 0.001
PE	0.87 ± 0.17	0.93 ± 0.28	N.S.	0.19 ± 0.03	< 0.005	0.23 ± 0.04	< 0.005
DG	0.47 ± 0.08	0.44 ± 0.10	N.S.	0.10 ± 0.02	< 0.005	0.08 ± 0.01	< 0.001
TG	1.39 ± 0.22	1.23 ± 0.16	N.S.	0.23 ± 0.03	< 0.001	0.41 ± 0.06	< 0.005
PA	1.08 ± 0.18	0.90 ± 0.08	N.S.	0.23 ± 0.08	< 0.005	0.24 ± 0.06	< 0.005
PI	1.12 ± 0.25	1.07 ± 0.20	N.S.	0.25 ± 0.03	< 0.01	0.31 ± 0.06	< 0.02
PPI	0.10 ± 0.01*	0.09 ± 0.01	N.S.	0.05 ± 0.01	< 0.001	0.06 ± 0.01	< 0.05

poor) concentration was varied according to that of fatty acids: 0.5, 1 or 2% (w/v) for 0 and 0.25, 0.5 or 1 mM-palmitate respectively.

The synthesis of islet lipids *de novo* was measured as the incorporation of D-[U-^{14}C]glucose after incubation for 120 min in the same medium (50 µl) as that used for secretion studies [2]. The uptake of $^{45}Ca^{2+}$ into isolated islets was measured with the La^{3+}-wash procedure [17], slightly modified as previously described in detail [2]. Islet contents of cyclic nucleotides (cAMP and cGMP) were measured with specific radioimmunoassays (^{125}I-RIA kits from New England Nuclear), as follows. Batches of 10 islets were preincubated for 30 min at 37 °C in 50 µl of the incubation medium used in secretion studies and containing 3 mM-glucose, with or without 1 mM-palmitate. Incubation (5 or 60 min) started after addition of prewarmed incubation medium (50 µl) containing the test substances. Pertussis toxin (3 µg/ml) was added during preincubation. After addition of 10 µl of 10 mM-caffeine, the tubes containing medium and islets were rapidly frozen in acetone chilled with solid CO_2. After thawing, the tubes were sonicated, heated for 5 min in a boiling water bath, and stored at −40 °C. Proteins were precipitated and cyclic nucleotides extracted with 0.8 ml of 80% (v/v) ethanol. After centrifugation, the supernatant was transferred to RIA tubes (500 µl for cGMP and 450 µl for cAMP) and evaporated at 37 °C. The dry residues were reconstituted with 0.1 ml of radioimmunoassay buffer. The calculated recovery of [^{3}H]cGMP was 96.4 ± 1.2% (n = 9). Efflux of cGMP into the incubation medium was checked by measuring it separately in islets and medium in some of the experiments. For this purpose, the tubes were centrifuged after addition of caffeine, and 90 µl of incubation medium was taken and processed as indicated above for the measurement of cGMP. The islets were then sonicated after addition of 100 µl of RIA buffer (0.05 M-sodium acetate, pH 6.2), and the resulting supernatant was used for the radioimmunological determination of cGMP. The cyclic nucleotide content of the incubation medium was low and changed slightly with time [3.9 ± 0.3 pmol/100 µl (n = 13) after 5 min; 5.6 ± 0.6 pmol/100 µl (n = 13) after 60 min; $P < 0.02$], but it was not modified when islet levels were elevated (results not shown).

Statistical comparisons between pairs of means were made with Student's *t* tests for non-paired values. A two-tailed $P < 0.05$ was considered statistically significant (N.S., not significant).

RESULTS

Insulin release

Table 1 shows the insulin-secretory response of isolated islets to 20 mM-glucose and how it is modified by palmitate and cyclic nucleotides. Glucose (20 mM) increased 6-fold the basal secretory rate (at 3 mM-glucose), and this response was significantly enhanced by 0.25 mM- and 1.0 mM-palmitate [11 ± 0.5 (n = 15) to

Table 3. Effect of 1 mM-8-Br-cGMP (cGMP) on the rate of incorporation of D-[U-^{14}C]glucose into phosphatidylcholine (PC), phosphatidylethanolamine (PE), diacylglycerols (DG), triacylglycerols (TG), phosphatidic acid (PA), phosphatidylinositol (PI) and polyphosphoinositides (PPI), in the absence of palmitate

Values denote means ± S.E.M.; five animals were used in each experimental condition, except where indicated (*n = 6). Statistical comparisons were performed by the non-paired Student's t test. Means were compared with the corresponding control in the absence of 8-Br-cGMP. Abbreviations: G3 and G20, 3 mM- and 20 mM-glucose respectively.

	D-[U-^{14}C]Glucose incorporation (pmol/2 h per islet)					
Addition(s)...	G3 (control)	G3+cGMP	P	G20 (control)	20+cGMP	P
PC	0.11±0.023	0.12±0.011	N.S.	0.43±0.08	0.32±0.08*	N.S.
PE	0.034±0.004	0.029±0.002	N.S.	0.08±0.007	0.08±0.02*	N.S.
DG	0.04±0.007	0.04±0.003	N.S.	0.22±0.11	0.11±0.04*	N.S.
TG	0.15±0.052	0.22±0.010	N.S.	0.58±0.19	0.51±0.10	N.S.
PA	0.03±0.003	0.04±0.005	N.S.	0.07±0.02	0.12±0.02*	N.S.
PI	0.04±0.003	0.11±0.016	N.S.	0.12±0.02	0.11±0.02*	N.S.
PPI	0.02±0.001	0.03±0.004	N.S.	0.03±0.003	0.03±0.004*	N.S.

Table 4. Effect of 8-bromo cyclic nucleoside monophosphates on the initial (15 min) uptake of $^{45}Ca^{2+}$ at either 3 mM- or 20 mM-glucose in the absence or presence of 1 mM-palmitate

Values denote means ± S.E.M., and the number of animals used in each experimental condition is shown in parentheses. Statistical comparisons were performed by the non-paired Student's t test. Abbreviations: 0.25cAMP and 1cAMP, 0.25 mM- and 1 mM-8-Br-cAMP respectively; 0.25cGMP, 0.25 mM-8-Br-cGMP; 0Palm and 1Palm, 0 and 1 mM-palmitate respectively; G3 and G20, 3 mM- and 20 mM-glucose respectively.

	15 min $^{45}Ca^{2+}$ uptake (pmol/islet)			
	0Palm	P	1Palm	P
G3	1.5±0.2 (3)	Control	1.8±0.2 (5)	Control
G3+0.25cAMP	2.3±0.3 (4)	N.S.	2.0±0.4 (5)	N.S.
G3+0.25cGMP	1.3±0.1 (4)	N.S.	2.0±0.2 (5)	N.S.
G3+0.25cGMP+1cAMP	–	–	2.0±0.1 (5)	N.S.
G20	4.7±0.1 (3)	Control	9.3±0.1 (5)	Control
G20+0.25cAMP	5.1±0.4 (4)	N.S.	9.5±0.8 (5)	N.S.
G20+0.25cGMP	4.5±0.4 (4)	N.S.	5.5±0.7 (5)	< 0.001
G20+0.25cGMP+1cAMP	–	–	6.3±0.4 (5)	< 0.001

Table 5. Effect of noradrenaline (NA) on total (islets + medium) cGMP content at different glucose concentrations (G3, 3 mM; G20, 20 mM) and in the absence (0Palm) or presence of 1 mM-palmitate (1Palm)

Values denote means ± S.E.M.; five animals were used in each experimental condition. Statistical comparisons were performed by the non-paired Student's t test with the corresponding controls in the absence of NA.

	cGMP content (fmol/10 islets)			
	0Palm	P	1Palm	P
G3	16.2±1.0	Control	16.1±1.0	Control
G3+10 nM-NA	18.6±0.4	N.S.	16.2±1.2	N.S.
G3+0.1 μM-NA	29.0±2.5	< 0.005	25.4±1.4	< 0.001
G3+1 μM-NA	49.4±2.8	< 0.001	45.6±1.5	< 0.001
G20	16.4±1.1	Control	14.9±0.7	Control
G20+10 nM-NA	17.4±1.5	N.S.	19.0±1.1	< 0.02
G20+0.1 μM-NA	31.1±3.0	< 0.02	26.2±1.6	< 0.001
G20+1 μM-NA	56.9±8.4	< 0.001	53.3±3.6	< 0.001

14±0.6 (n = 6; P < 0.01) and 24±0.8 ng/h per islet (n = 18, P < 0.001)]. Increasing concentrations of 8-Br-cAMP augmented significantly and dose-dependently the response to glucose at 0

Table 6. Effect of noradrenaline (NA) on the islet cGMP content after 5 or 60 min of incubation

Values denote means ± S.E.M.; five animals were used in each experimental condition, except where indicated (*n = 4). Statistical comparisons were performed by the non-paired Student's t test with the corresponding controls in the absence of NA.

		cGMP content (fmol/10 islets)			
Glucose (mM)	NA (μM)	5 min	P	60 min	P
3	–	5.7±1.4	Control	11.5±2.2	Control
3	0.1	11.0±2.5	N.S.	27.7±2.1	< 0.001
3	1.0	13.0±1.8	< 0.01	41.6±4.3	< 0.001
20	–	6.1±0.6	Control	9.2±1.3	Control
20	0.1	14.8±2.4	< 0.005	29.4±3.8	< 0.001
20	1.0	12.0±1.5*	< 0.005	46.3±5.2	< 0.001

and 0.25 μM-palmitate, but not at 1 mM. The absolute increase induced by 1 mM-8-Br-cAMP was very similar at 0 and 0.25 mM-palmitate (5.1 and 6.1 ng of insulin/h per islet). On the contrary, increasing concentrations of 8-Br-cGMP significantly decreased the insulin response to glucose in the absence and presence of palmitate. In its absence, the maximum effect was modest

Table 7. Stimulation by nitroprusside of total (islets + medium) cGMP content after 5 or 60 min of incubation

Values denote means ± S.E.M.; the number of animals used in each experimental condition is shown in parentheses. Statistical comparisons were performed by the non-paired Student's *t* test with the corresponding controls in the absence of nitroprusside.

Nitroprusside (μM)	cGMP content (fmol/10 islets)			
	5 min	*P*	60 min	*P*
–	8.9 ± 0.9 (6)	Control	16.2 ± 1.5 (5)	Control
10	14.2 ± 0.9 (5)	< 0.005	24.7 ± 2.8 (5)	N.S.
100	31.9 ± 4.5 (6)	< 0.001	63.1 ± 8.3 (6)	< 0.001

(−27%) and only recorded at high 8-Br-cGMP concentrations; in its presence, a greater decrease was obtained (−41% and −54% at 0.25 mM- and 1.0 mM-palmitate respectively), which was clearly dose-dependent at 1 mM-palmitate. 8-Br-cGMP (0.25 mM) decreased by approx. 50% the enhancing effect of 1 mM-palmitate on glucose-induced insulin response in either the absence or the presence of 1 mM-8-Br-cAMP [16 ± 0.7 ($n = 15$) versus 18 ± 1.2 ng/h per islet ($n = 8$); N.S.].

Lipid synthesis *de novo*

As shown in Table 2, the incorporation of 20 mM-D-[U-^{14}C]glucose into the different classes of islet lipids at 1.0 mM-palmitate was significantly decreased (by 77–84% in most of them and by 53% in polyphosphoinositides) by 1 mM-8-Br-cGMP. This decrease was not significantly modified by the simultaneous presence of 1 mM-8-Br-cAMP, except for triacylglycerols, which incorporated more labelled glucose in the presence of both nucleotides [0.4 ± 0.06 ($n = 6$) versus 0.2 ± 0.03 pmol/h per islet ($n = 6$); $P < 0.02$). 8-Br-cAMP alone had no effect on the rate of incorporation of labelled glucose into any of the lipid fractions. The rate of D-[U-^{14}C]glucose incorporation into each of the islet lipid fractions was lower in the absence of palmitate, and it was not significantly affected by 1 mM-8-Br-cGMP (Table 3).

$^{45}Ca^{2+}$ uptake

The initial (15 min) uptake of $^{45}Ca^{2+}$ was stimulated either 3- or 5-fold by 20 mM-glucose in the absence or presence of 0.25 mM-palmitate, respectively (Table 4). 8-Br-cAMP (1 mM) did not significantly modify this initial rate of $^{45}Ca^{2+}$ uptake at any glucose and/or palmitate concentration. On the contrary, 8-Br-cGMP (0.25 mM) decreased glucose (20 mM)-induced $^{45}Ca^{2+}$ uptake in the presence of 1 mM-palmitate to the levels recorded in its absence [5.5 ± 0.7 ($n = 5$) versus 4.7 ± 0.1 pmol/islet ($n = 3$); N.S.) without modifying the effect of glucose alone. This decrease was not significantly affected by addition of 1 mM-8-Br-cAMP.

Islet content of cyclic nucleotides

Noradrenaline increased dose-dependently the total content (islets + medium) of cGMP (Table 5) as well as that of islets when it was measured separately (Table 6). Nitroprusside, a known activator of soluble guanylate cyclase [18], also increased dose-dependently the islet content of cGMP, which was already significantly elevated after 5 min of incubation (Table 7).

Islet cGMP content was not affected by 10 μM-yohimbine, which, however, completely prevented the increase induced by noradrenaline (1 μM) after 5 or 60 min of incubation (Table 8). Pretreatment with pertussis toxin (3 μg/ml) also abolished the noradrenaline-induced increase in islet cGMP content.

Table 8. Effect of 1 μM-noradrenaline (NA) on total (islets + medium) content of both cGMP and cAMP after 5 or 60 min of incubation and its modification by either 10 μM-yohimbine (Y) or pretreatment with 3 μg of pertussis toxin/ml (PT)

Values denote means ± S.E.M.; the number of animals used in each experimental condition is given in parentheses. Statistical comparisons were performed by the non-paired Student's *t* test.

Glucose (mM)	NA	Y	PT	cGMP content (fmol/10 islets)			
				5 min	*P*	60 min	*P*
3	–	–	+	8.9 ± 0.9 (6)	Control	16.2 ± 1.5 (5)	Control
3	+	–	–	13.3 ± 1.3 (5)	< 0.02	49.4 ± 2.8 (5)	< 0.001
3	–	+	–	9.9 ± 0.5 (4)	N.S.	–	–
3	+	+	–	9.3 ± 0.9 (6)	N.S.	–	–
20	–	–	–	7.5 ± 0.7 (5)	Control	16.4 ± 1.1 (5)	Control
20	+	–	–	14.0 ± 1.8 (5)	< 0.02	56.9 ± 2.4 (5)	< 0.001
20	–	+	–	9.3 ± 1.2 (5)	N.S.	15.6 ± 2.1 (5)	N.S.
20	+	+	–	9.5 ± 0.6 (5)	N.S.	12.9 ± 1.0 (6)	N.S.
20	–	–	+	8.9 ± 0.6 (7)	N.S.	15.5 ± 1.6 (8)	N.S.
20	+	–	+	9.0 ± 0.7 (8)	N.S.	15.8 ± 1.6 (8)	N.S.

Glucose (mM)	NA	Y	PT	cAMP content (pmol/10 islets)			
				5 min	*P*	60 min	*P*
3	–	–	–	0.28 ± 0.02 (5)	< 0.005	–	–
20	–	–	–	0.64 ± 0.09 (5)	Control	0.38 ± 0.05 (3)	Control
20	+	–	–	0.35 ± 0.03 (4)	< 0.05	0.30 ± 0.03 (2)	N.S.
20	–	+	–	0.69 ± 0.08 (4)	N.S.	0.39 ± 0.06 (4)	N.S.
20	+	+	–	0.52 ± 0.03 (6)	N.S.	0.35 ± 0.01 (4)	N.S.
20	–	–	+	0.85 ± 0.2 (5)	N.S.	0.46 ± 0.07 (7)	N.S.
20	+	–	+	0.49 ± 0.04 (7)	N.S.	0.40 ± 0.03 (5)	N.S.

Islet levels of cAMP were significantly elevated by 20 mM-glucose after 5 min of incubation, but they were again at basal values after 60 min (Table 8). Noradrenaline (1 μM) blocked the response to glucose, and this effect was only partially reversed by 10 μM-yohimbine or pretreatment with pertussis toxin. Nitroprusside did not significantly modify islet cAMP content (results not shown).

DISCUSSION

The results of the present work strongly suggest that the bromo derivative of cGMP inhibits dose-dependently the potentiation by palmitate of the insulin response to glucose. The proper effect of glucose is much less sensitive to the cyclic nucleotide; it is only slightly inhibited by high concentrations. cGMP has been shown to potentiate dose-dependently, in the range 1–5 mM, the glucose-induced insulin response of islets in a 'starved state' [19] or from starved rats [20], but it has no effect in islets from fed animals [20]. On the contrary, it has been suggested that the cGMP system may be inhibitory in some secretory cells [18,21,22]. Moreover, it has also been shown that dibutyryl cGMP inhibits the potentiation of glucose-induced insulin release by cholecystokinin in the perfused rat pancreas [23].

The inhibitory action of 8-Br-cGMP (0.25 mM) is not affected by 8-Br-cAMP (1 mM), which potentiates, as expected [4], the insulin response to glucose in an apparently additive manner with palmitate. Therefore, even if the two cyclic nucleotides exert opposite effects on secretion, as was generically postulated by the old 'Yin Yang' hypothesis [24], they do not directly antagonize each other by the same mechanism (palmitate potentiation of insulin secretion).

The mechanism underlying the inhibition of palmitate potentiation of insulin release by 8-Br-cGMP seems to involve its ability to block islet lipid synthesis *de novo* and the $^{45}Ca^{2+}$ turnover, for several reasons. First, this nucleotide analogue inhibits specifically the potentiation by palmitate of labelled glucose incorporation into islet lipids without modifying the proper effect of an increase of glucose concentration. Second, 8-Br-cGMP partially inhibits the stimulation of islet $^{45}Ca^{2+}$ uptake induced by glucose and palmitate, but not that caused by glucose alone. And third, the restoration by fatty-acid-oxidation inhibitors of lipid synthesis [3], $^{45}Ca^{2+}$ turnover [5] and insulin secretion [3] in starved islets stimulated by glucose and palmitate speaks in favour of priority of lipid metabolism in the sequence of events leading to palmitate potentiation of glucose-stimulated secretion.

The islet content of cGMP is increased dose-dependently by noradrenaline with α_2-adrenergic specificity, but it is not modified by glucose and/or palmitate. Previous studies have demonstrated that glucose, alone [25,26] or together with a phosphodiesterase inhibitor [27,28], increases moderately islet cGMP levels. However, a several-fold higher elevation of islet cGMP by either nitroprusside [25] or atrial natriuretic peptide [29] resulted in an inhibited or unmodified insulin response to glucose or glyceraldehyde, respectively. Within this context, it is not easy to understand that exogenous cGMP can partially counteract the inhibition of glucose-induced secretion caused by noradrenaline (10 μM) in rat islets [30]. The presence of a particulate form of guanylate cyclase was first demonstrated in islet homogenates by Howell & Montague [27], who found that adrenaline and noradrenaline inhibited its activity at higher doses (10 μM) than those used in the present study. We also consider that results obtained in islet homogenates cannot be directly extrapolated to whole islets, or vice versa, owing to obvious differences in experimental design.

The suppression of noradrenaline-induced stimulation of cGMP by pertussis toxin strongly suggests that islet α_2-adrenoceptor may be positively coupled to guanylate cyclase through a G_i-like protein. A membrane-associated form of guanylate cyclase that specifically binds atrial natriuretic peptides has been demonstrated in human and rat tissues [31]. There is also evidence that atrial-natriuretic-factor receptors are coupled to adenylate cyclase through an inhibitory G-protein [32]. Rat insulinoma cells also possess a specific receptor for atrial natriuretic peptide, and its activation increases cGMP levels without affecting the insulin response to glyceraldehyde [29]. However, atrial natriuretic factor inhibits the glucose-induced increase in plasma insulin levels in the rat [33]. The dose-dependent stimulation of islet cGMP content by nitroprusside is also evidence for the existence in islet cells of a soluble form [31] of guanylate cyclase whose regulation probably differs from that of the membrane enzyme.

Stimulation of the islet cAMP system by glucose and its inhibition by noradrenaline with α_2-adrenergic specificity has been known for a long time [4], and it could also be demonstrated in the present work. Therefore, α_2-adrenoceptor stimulation induces in islets, as in adrenocortical carcinoma cells [15], a dual regulation of both adenylate and guanylate cyclases, which are respectively inhibited and activated. The resultant elevation of cGMP levels may mediate the inhibition of islet lipid synthesis *de novo* [5] and, consequently, the suppression of the accelerating effect of palmitate on glucose-induced $^{45}Ca^{2+}$ turnover and insulin release [5]. Inhibition by noradrenaline of the proper effect of glucose is then probably mediated by different mechanisms [6,7].

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