Biochemical Pharmacology, Vol. 53, pp. 1211–1213, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(96)00823-4

SHORT COMMUNICATION

3-Morpholinosydnonimine as Instigator of a Glibenclamide-Sensitive Reduction in the Insulin Secretory Rate

*Marie-Helene Antoine, Raogo Ouedraogo, Marcel Hermann, Jacqueline Sergooris, Andre Herchuelz and Philippe Lebrun**

LABORATORY OF PHARMACOLOGY, UNIVERSITÉ LIBRE DE BRUXELLES, SCHOOL OF MEDICINE, 808 ROUTE DE LENNIK, B-1070 BRUSSELS, BELGIUM

ABSTRACT. The nitric oxide (NO) donor SIN-1 (3-morpholinosydnonimine) induced a concentration-dependent inhibition of the secretory response to glucose. The negative insulinotropic action of SIN-1 was attenuated by the hypoglycemic sulfonylurea glibenclamide. Moreover, the NO donor enhanced ^{86}Rb outflow from perfused islets and reduced the glucose-induced increase in ^{45}Ca outflow. The present data provide further evidence that NO donors impair the secretory response to glucose, at least in part, by activating the ATP-sensitive K^+ channels. BIOCHEM PHARMACOL 53;8:1211–1213, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. SIN-1; glibenclamide; insulin release; pancreatic islets

NO is an important biological messenger responsible for regulating a wide range of cellular functions, and has been recognized as the endogenous stimulator of the soluble guanylate cyclase [1]. Recent findings have indicated that exposure of insulin-secreting cells to SIN-1, a NO generator, increased cGMP concentrations, but impeded the secretory capacity of glucose [2–4]. Because modifications in both K^+ and Ca^{2+} permeabilities are known to represent critical events in glucose-induced insulin release, we determined whether or not the secretory response to SIN-1 involves interference with transmembrane cationic movements.

MATERIALS AND METHODS

All experiments were performed with islets isolated from the pancreas of fed Wistar rats. The methods used to measure insulin release from incubated islets as well as ^{86}Rb (^{42}K substitute) efflux, ^{45}Ca efflux, and insulin release from perifused pancreatic islets have been previously described [5–7]. For measurement of insulin secretion from incubated islets, the experiments were repeated 3 times on different days with the values corresponding to the number of samples pooled. The outflow of ^{86}Rb or ^{45}Ca (counts/min/min) was expressed as a FOR (percentage of instantaneous islet content per min). Results are expressed as mean values (± SEM) together with the number of individual experiments. The statistical significance of differences between mean values was assessed by use of Student's *t*-test or analysis of variance. SIN-1 was obtained from Therabel Research (Brussels, Belgium), SNAP from Wellcome (Erembodegem, Belgium) and glibenclamide from Hoechst Roussel (Brussels, Belgium). The experiments were conducted with minimal light to prevent photodegradation of the compounds.

RESULTS AND DISCUSSION

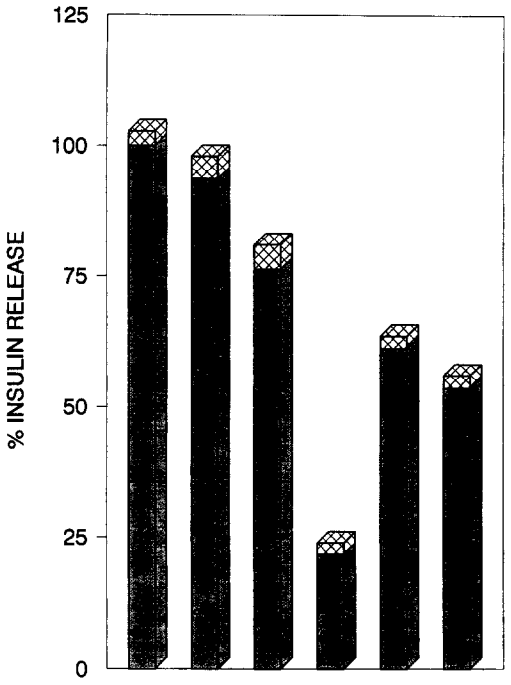
Addition of SIN-1 to islets exposed to 16.7 mM glucose provoked a concentration-dependent reduction in insulin output ($R = 0.972$; $P < 0.05$) (Fig. 1). Under the same experimental conditions, high concentrations of the NO donor SNAP also reduced glucose-induced insulin release. After addition of 1 mM and 5 mM SNAP, the residual insulin release averaged 91.5 ± 2.8% (NS) and 67.4 ± 3.3% of the control experiments ($P < 0.05$), respectively (data not shown). Figure 1 further reveals that the presence of glibenclamide in the incubation medium attenuated the negative insulinotropic action of SIN-1. Thus, the residual insulin release averaged 21.8 ± 2.1% after the addition of 1 mM SIN-1 whereas, in the simultaneous presence of 1 mM SIN-1 and 10 or 50 μM glibenclamide, the insulin output represented 61.3 ± 2.3% and 53.6 ± 2.5% of the control values (no added drug) ($P < 0.05$).

The present data further provide evidence that nitric oxide donors impede the insulinotropic action of D-glucose [3, 4, 7]. Moreover, the presence of the hypoglycemic sulfonylurea glibenclamide in the incubation medium was shown to reduce the inhibitory effect of SIN-1 on the insulin-releasing process. Because glibenclamide is known to

* Corresponding author. P. Lebrun, Laboratory of Pharmacology, Université Libre de Bruxelles, School of Medicine (CP 617), 808 route de Lennik, B-1070 Brussels, Belgium. Tel: 32-2-555 62 02; FAX 32-2-555 63 70.

† *Abbreviations:* NO, nitric oxide; FOR, fractional outflow rate; SIN-1, 3-morpholinosydnonimine; SNAP, S-nitroso-N-acetylpenicillamine.

Received 7 May 1996; accepted 8 October 1996.



GLUCOSE	(mM)	16.7	16.7	16.7	16.7	16.7	16.7
SIN-1	(M)	0	10^{-5}	10^{-4}	10^{-3}	10^{-3}	10^{-3}
GLIBENCLAMIDE	(M)	0	0	0	0	10^{-6}	10^{-5}

FIG. 1. Effect of increasing concentrations of SIN-1 on insulin release from islets incubated in the presence of 16.7 mM glucose and in the presence or absence of glibenclamide. Insulin release was expressed in percent of the value recorded in control experiments (100%, no added drug and presence of 16.7 mM glucose). Mean values (+ SEM) refer to 16–35 samples.

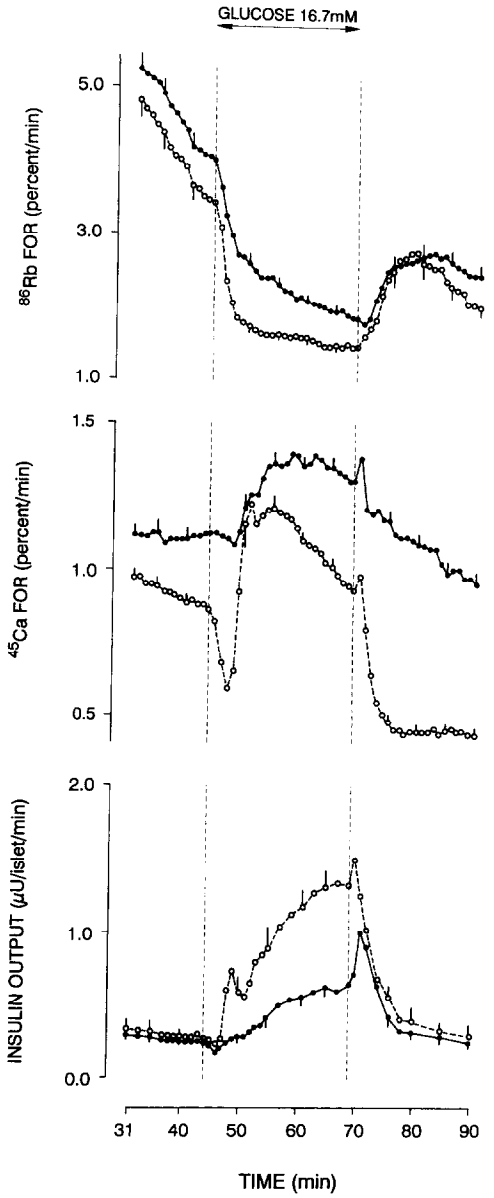


FIG. 2. Effect of glucose (16.7 mM) on ^{86}Rb FOR (top panel), ^{45}Ca FOR (middle panel), and insulin release (bottom panel) from islets perifused throughout in the absence (○) or presence (●) of SIN-1 (500 μM). Basal media contained Ca^{2+} (2.56 mM). Mean values (± SEM) refer to 7–10 individual experiments.

block the ATP-sensitive K^+ channels of pancreatic B-cells [8], such findings suggest that the negative secretory action of SIN-1 may be attributable to an increase in membrane K^+ permeability. ATP-sensitive K^+-channel activation may be expected to restrict the opening of voltage-sensitive Ca^{2+} channels, decrease Ca^{2+} entry and, in turn, to inhibit insulin output. Radioisotopic and dynamic measurements conducted on perfused rat pancreatic islets substantiated, although indirectly, this hypothesis. First, SIN-1 (500 μM) increased the rate of ^{86}Rb outflow from pancreatic islets perifused in the presence of 5.6 mM glucose (data not shown). Moreover, in islets exposed to SIN-1 (500 μM) throughout, the inhibitory effect of glucose (16.7 mM) on the ^{86}Rb outflow rate was less marked than in control experiments (Fig. 2, upper panel.) The mean value of ^{86}Rb FOR recorded after 16 to 25 min of exposure to glucose averaged 1.98 ± 0.05%/min in the presence and 1.51 ± 0.05%/min in the absence of SIN-1 in the perifusate ($P < 0.001$). These changes in ^{86}Rb FOR can be interpreted as being due to an increase in membrane K^+ permeability, itself resulting from a weaker inhibitory effect of glucose on the ATP-sensitive K^+ channels. Second, when the perifusate was enriched with SIN-1 (500 μM), the increment in ^{45}Ca outflow induced by glucose (16.7 mM) was reduced, only representing 42.3 ± 3.9% of that evoked by the sugar in the absence of the NO donor ($P < 0.005$) (Fig. 2, middle panel). This inhibitory effect of SIN-1 on the increase in ^{45}Ca FOR reflects a reduction in glucose-induced Ca^{2+} entry into the islet cells. Indeed, the stimulatory effect of glucose on ^{45}Ca FOR has been ascribed to a process of sustained exchange between influent ^{40}Ca and effluent ^{45}Ca [5, 9]. Incidentally, the basal (min 31–44) ^{45}Ca outflow was higher in the presence than in the absence of SIN-1 in the physiological medium ($P < 0.05$). Such a

feature can reflect an intracellular redistribution of Ca^{2+} ions [6], a phenomenon that is substantiated by the recent finding that aqueous NO can provoke Ca^{2+} mobilization in insulin-secreting cells [10]. Finally, the simultaneous measurement of ^{45}Ca FOR and insulin release indicates that the presence of SIN-1 (500 μM) in the perfusing medium impaired the secretory response to glucose (Fig. 2, lower panel). The integrated output of insulin measured during exposure to 16.7 mM glucose averaged 48.4 ± 6.5% of that recorded in control experiments ($P < 0.005$).

This cascade of events initiated by modifications in ATP-sensitive K^+-channel activity and culminating in the inhibition of insulin output has also been described for another NO generator, namely sodium nitroprusside [7, 11]. The precise mechanism(s) by which SIN-1 interferes with the activity of ATP-sensitive K^+ channels remain(s) unclear. However, the cationic responses to NO donors might result from alterations of the glycolytic pathway [11].

Although our findings indicate that direct or indirect activation of ATP-sensitive K^+ channels may underlie the negative insulinotropic action of SIN-1, additional mechanisms appear to be involved in the NO donor modulation of the secretory process. Indeed, glibenclamide attenuated but did not fully reverse the secretory response to SIN-1. An increase in intracellular cGMP concentration, as provoked by SIN-1 [2–4], is unlikely to mediate the glibenclamide-resistant inhibitory effect of the NO donor, because exogenous dibutyryl cGMP has previously been shown to potentiate glucose-induced insulin release without affecting transmembrane cationic movements [7]. The glibenclamide-resistant negative insulinotropic action of SIN-1 may be viewed, "inter alia," as the result of NO-induced DNA damage [4] or of modifications in the intracellular cAMP and/or GTP content [3, 4]. The present findings, however, cannot exclude the possibility that a direct inhibitory effect of SIN-1 on the voltage-dependent Ca^{2+} channels could mediate the hypoglycemic sulfonylurea-resistant inhibition of the secretory process.

In conclusion, the present data provide further evidence that NO donors impair the secretory response to glucose, at least in part, by activating ATP-sensitive K^+ channels.

The authors are grateful to P. Surardt for secretarial help. This study was supported in part by a grant from the National Fund for Scientific Research (Belgium) where P. Lebrun is a Senior Research Associate. R. Ouedraogo was supported by a grant-in-aid from the Université Libre de Bruxelles and from Burkina Faso.

References

1. Moncada S, Palmer RMJ and Higgs EA, Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol Rev **43:** 109–142, 1991.
2. Schmidt HHHW, Warner TD, Ishii K, Sheng H and Murad F, Insulin secretion from pancreatic B cells caused by L-arginine-derived nitric oxides. Science **255:** 721–723, 1992.
3. Cunningham JM, Mabley JG, Delaney CA and Green IC, The effect of nitric oxide donors on insulin secretion, cyclic GMP and cyclic AMP in rat islets of Langerhans and the insulin-secreting cell lines HIT-15 and RINm5F. Mol Cell Endocrinol **102:** 23–29, 1994.
4. Green IC, Cunningham JM, Delaney CA, Elphick MR, Mabley JG and Green MHL, Effects of cytokines and nitric oxide donors on insulin secretion, cyclic GMP and DNA damage: relation to nitric oxide production. Biochem Soc Trans **22:** 30–37, 1994.
5. Lebrun P, Malaisse WJ and Herchuelz A, Evidence for two distinct modalities of Ca^{2+} influx into pancreatic B cell. Am J Physiol **242:** E59–E66, 1982.
6. Lebrun P, Malaisse WJ and Herchuelz A, Nutrient-induced intracellular calcium movement in rat pancreatic B-cell. Am J Physiol **243:** E196–E205, 1982.
7. Antoine MH, Hermann M, Herchuelz A, and Lebrun P, Sodium nitroprusside inhibits glucose-induced insulin release by activating ATP-sensitive K^+ channels. Biochim Biophys Acta **1175:** 293–301, 1993.
8. Malaisse WJ and Lebrun P, Mechanisms of sulfonylurea-induced insulin release. Diabetes Care 13, suppl **3:** 9–17, 1990.
9. Herchuelz A, Couturier E and Malaisse WJ, Regulation of calcium fluxes in pancreatic islets: glucose-induced calcium-calcium exchange. Am J Physiol **238:** E96–E103, 1980.
10. Willmott NJ, Galione A and Smith PA, Nitric oxide induces intracellular Ca^{2+} mobilization and increases secretion of incorporated 5-hydroxytryptamine in rat pancreatic B-cells. Febs Lett **371:** 99–104, 1995.
11. Tsuura Y, Ishida H, Hayashi S, Sakamoto K, Horie M and Seino Y, Nitric oxide opens ATP-sensitive K^+ channels through suppression of phosphofructokinase activity and inhibits glucose-induced insulin release in pancreatic B-cells. J Gen Physiol **104:** 1079–1099, 1994.