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Hydroxylamine, a nitric oxide donor, inhibits insulin release and activates K^+_{ATP} channels

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Abstract

The present study was undertaken to assess the effects of hydroxylamine, a nitric oxide (NO) donor, on ionic and secretory events in rat pancreatic islets. Hydroxylamine provoked a concentration-dependent inhibition of the glucose-induced insulin release. This inhibitory action was counteracted by glibenclamide. Moreover, hydroxylamine increased the rate of ^{86}Rb outflow from perifused islets. This effect persisted in the absence of external Ca^{2+} but was impaired by glibenclamide. Hydroxylamine decreased ^{45}Ca outflow, $[Ca^{2+}]_i$ and insulin output from islets exposed to 16.7 mM glucose and extracellular Ca^{2+}. By contrast, hydroxylamine did not affect the increase in ^{45}Ca outflow and $[Ca^{2+}]_i$ evoked by K^+ depolarization. These experimental results suggest that the negative insulinotropic action of the NO donor results, at least in part, from the activation of ATP-sensitive K^+ channels leading to a decrease in Ca^{2+} influx and $[Ca^{2+}]_i$. Additional mechanisms, however, could also be involved in the NO donor modulation of the secretory process.

Keywords: Pancreatic islet, rat; Insulin release; Nitric oxide (NO); Hydroxylamine; K^+ channel, ATP-sensitive

1. Introduction

Nitric oxide (NO) has been shown to be involved in numerous biological processes including vascular smooth muscle relaxation, neurotransmission, platelet agregation, immunological reactions, penile erection, exocrine and endocrine function, etc. (Moncada et al., 1991; Schmidt and Walter, 1994). It is now becoming apparent that NO is implicated in diverse physiological roles and that almost all mammalian cells are under its influence.

Insulin-secreting cells appear to be equipped with a constitutive, Ca^{2+}-sensitive, NO synthase which can produce NO from the amino acid L-arginine (Green et al., 1994; Schmidt et al., 1992). Macrophages and endothelial cells, which possess an inducible, Ca^{2+}-independent, form of NO synthase, can generate large amounts of NO after cytokines activation (Moncada et al., 1991). Hence, both isoforms of NO synthase can conceivably play a role in the control of the insulin-releasing process. Recent studies have suggested the involvement of NO in pancreatic B-cell function (Schmidt et al., 1992; Schmidt and Walter, 1994) and have also demonstrated that the inhibitory effects of cytokines on insulin-secreting cells could be related to the generation of NO (Cunningham et al., 1994; Green et al., 1993, 1994; Southern et al., 1990). Few investigations, however, have addressed the question whether modifications in transmembrane ionic movements might play a role in the physiological response to NO.

In order to further document the effects of NO on the secretory sequence and to evaluate the putative contribution of changes in ionic fluxes, we have characterized the effects of hydroxylamine on insulin release, radioisotopic fluxes and cytosolic Ca^{2+} concentration in rat pancreatic B-cells. Hydroxylamine is known as a precursor of NO which penetrates easily into cells (DeMaster et al., 1989). Although nitroprusside and sydnonimines can give rise to NO through non-enzymatic processes, the NO generation from hydroxylamine requires a catalase-dependent reaction (Craven et al., 1979; DeMaster et al., 1989). Thus, hydroxylamine can be viewed as an intracellular generator of NO and this compound might be expected to mimic more closely the events following an increase in intracellular NO production (Green et al., 1994; Mabley et al., 1993).

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2. Materials and methods

2.1. Animals

All experiments were performed with pancreatic islets isolated by the collagenase method from fed female albino rats.

2.2. Measurements of insulin release from incubated islets

For the measurement of insulin secretion from incubated islets, groups of 10 islets, each derived from the same batch of islets, were pre-incubated for 30 min at 37°C in 1 ml of a bicarbonate-buffered solution (in mM: NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24) supplemented with 2.8 mM glucose, 0.5% (w/v) dialysed albumin (fraction V; Sigma, St. Louis, MO, USA) and equilibrated against a mixture of O_2 (95%) and CO_2 (5%). The islets were then incubated at 37°C for 90 min in 1 ml of the same medium containing 16.7 mM glucose and, in addition, either hydroxylamine, sodium nitroprusside or glibenclamide. Experiments were repeated 3 times, on different days. The release of insulin was measured radioimmunologically using rat insulin as a standard (Leclercq-Meyer et al., 1975).

2.3. Measurements of ^{86}Rb, ^{45}Ca outflow and insulin release from perifused islets

The methods used to measure ^{86}Rb (^{42}K substitute) efflux, ^{45}Ca efflux and insulin release from perifused islets have been described in prior publications (Antoine et al., 1993; Lebrun et al., 1982a,b). Briefly, groups of 100 islets were incubated for 60 min in a bicarbonate-buffered medium containing 16.7 mM glucose and either ^{86}Rb (0.15–0.25 mM; 50 μCi/ml) or ^{45}Ca (0.02–0.04 mM; 100 μCi/ml). After incubation, the islets were washed 3 times and then placed in a perifusion chamber. The perifusate was delivered at a constant rate (1.0 ml/min). From the 31st to the 90th min, the effluent was continuously collected over successive periods of 1 min each. An aliquot of the effluent (0.5 ml) was used for scintillation counting while the remainder was stored at −20°C for insulin radioimmunoassay. At the end of the perifusion, the radioactive content of the islets was also determined. The outflow of ^{86}Rb or ^{45}Ca (cpm/min) was expressed as a fractional outflow rate (% of instantaneous islet content/min). The validity of ^{86}Rb as a tracer for the study of K^+ handling in the islets has been previously assessed (Malaisse et al., 1978).

2.4. Measurements of fura 2 fluorescence from single islet cells

The islets were disrupted in a Ca^{2+}-deprived medium and then centrifuged through an albumin solution to remove debris and dead cells. The cells were seeded onto glass coverslips and maintained in tissue culture during 72 h before use. Islet cells were cultured in RPMI 1640 culture medium (GIBCO, Europe) supplemented with 10% (v/v) newborn calf serum and containing glutamine (2.3 mM), glucose (16.7 mM), penicillin G (100 IU/ml) and streptomycin (100 μg/ml). The cells were then incubated with Fura-2 AM (2 μM) (Molecular Probes, Eugene, OR, USA) for 1 h and, after washing, the coverslips with the cells were mounted as the bottom of an open chamber placed on the stage of the microscope. The medium used to perfuse the cells contained (in mM): NaCl 115, KCl 5, $CaCl_2$ 2.56, $MgCl_2$ 1, $NaHCO_3$ 24, glucose 2.8 and was gassed with O_2 (95%)/CO_2 (5%). Fura-2 fluorescence of loaded cells was measured by dual-excitation microfluorimetry using a camera-based system (Applied Imaging, UK). The excitation and emission wavelengths were set at 340/380 and 510 nm, respectively. All experiments were repeated at least 3 times, on different days. The trace shown is representative of one coverslip containing 19 cells.

2.5. Drugs

In some experiments, extracellular Ca^{2+} was eliminated by the removal of $CaCl_2$ and the addition of 0.5 mM EGTA (Sigma). Different media also contained (as required) glucose (Merck, Darmstadt, Germany), hydroxylamine (Merck), sodium nitroprusside (Aldrich Chemie, Belgium) and glibenclamide (Upjohn, Kalamazoo, MI, USA). Glibenclamide was dissolved in dimethylsulfoxide which was added to both control and test media at final concentrations not exceeding 0.1% (v/v). When high concentrations of K^+ were used, the concentration of NaCl was lowered in order to keep the osmolarity constant.

2.6. Calculations

Results are expressed as the mean (±S.E.M.). The magnitude of the increase in ^{86}Rb and ^{45}Ca outflow was estimated in each individual experiment from the integrated outflow of ^{86}Rb or ^{45}Ca observed during stimulation (45–68th min) after correction for basal value (40–44th min). Peak ^{45}Ca outflow was estimated from the difference in ^{45}Ca outflow between the highest value recorded during stimulation and the mean basal value (40–44th min) found within the same experiment. The inhibitory effect of hydroxylamine on ^{45}Ca outflow and insulin release from islets perifused in the presence of 16.7 mM glucose was taken as the difference between the mean value for ^{45}Ca outflow or insulin output recorded in each individual experiment between the 40–44th and 60–68th min of perifusion. The statistical significance of differences between mean data was assessed by using Student's *t*-test or by analysis of variance followed for multiple comparisons by a Scheffé test procedure.

3. Results

3.1. Effects of hydroxylamine on insulin release from incubated islets

A rise in the extracellular concentration of glucose from 2.8 to 16.7 mM elicited a marked increase in insulin output from incubated islets ($P < 0.05$) (Fig. 1). The addition of hydroxylamine to islets incubated in the presence of 16.7 mM glucose provoked a concentration-dependent decrease in insulin release (Fig. 1). Thus, after the addition of 10 μM, 100 μM, 1 mM and 10 mM hydroxylamine, the residual insulin release averaged 86.1 ± 5.8 (not significant), 59.7 ± 3.4, 11.5 ± 0.8 and 7.5 ± 0.7% of the control value ($P < 0.05$), respectively.

Fig. 2 illustrates the effects of 500 μM hydroxylamine on insulin release from islets incubated in the presence of 16.7 mM glucose and in the absence or presence of increasing concentrations of glibenclamide. The data revealed that the hypoglycemic sulfonylurea reduced but did not completely reverse the inhibitory effect of the NO donor. Indeed, the residual insulin release averaged 12.1 ± 1.0% ($P < 0.05$) after the addition of 500 μM hydroxylamine, whereas, in the simultaneous presence of 500 μM hydroxylamine and 100 nM, 1 μM, 10 μM or 50 μM glibenclamide, the insulin output averaged 57.1 ± 3.3, 63.8 ± 5.4, 51.4 ± 2.9 and 53.1 ± 4.2% of the control experiments (no added drug) ($P < 0.05$).

In islets exposed simultaneously to 1 mM hydroxylamine and 10 μM glibenclamide, insulin release represented 49.7 ± 2.3% of the insulin output recorded in the presence of 16.7 mM glucose ($P < 0.05$; data not shown).

We next explored the effects of glibenclamide on the decrease in insulin secretion brought about by sodium nitroprusside. Sodium nitroprusside, another NO donor, was previously shown to induce a dose-related reduction

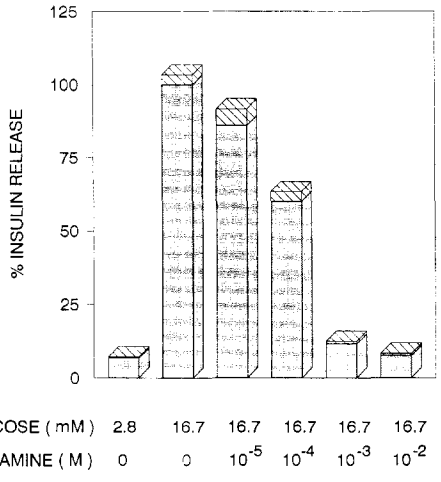

Fig. 1. Effect of increasing concentrations of hydroxylamine on insulin release from islets incubated in the presence of 16.7 mM glucose. Insulin release was expressed in percentage of the value recorded in control experiments (100%; no added drug and presence of 16.7 mM glucose). Mean values (±S.E.M.) refer to 14–35 samples.

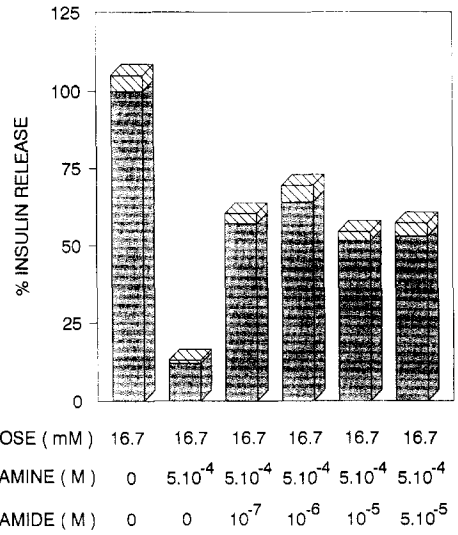

Fig. 2. Effect of increasing concentrations of glibenclamide on insulin release from islets incubated in the presence of 16.7 mM glucose and 5.10^{-4} M hydroxylamine. Insulin release was expressed in percentage of the value recorded in control experiments (100%, no added drug and presence of 16.7 mM glucose). Mean values (±S.E.M.) refer to 8–30 samples.

of the glucose-induced insulin release (Antoine et al., 1993). In the presence of 16.7 mM glucose and 500 μM sodium nitroprusside in the incubation medium, the release of insulin averaged 12.8 ± 0.7% of that recorded in the presence of glucose (16.7 mM) but absence of drug ($P < 0.05$; data not shown). The further addition of glibenclamide counteracted, at least in part, the inhibitory effect of sodium nitroprusside. After the addition of 10 and 50 μM glibenclamide, the insulin output represented 55.5 ± 2.7 and 43.0 ± 2.4% of the control value, respectively ($P < 0.05$; data not shown).

3.2. Effects of hydroxylamine on ^{86}Rb and ^{45}Ca outflow from islets perifused in the absence or presence of 5.6 mM glucose

Whether the physiological medium contained or was deprived of extracellular Ca^{2+}, the addition of hydroxylamine (1 mM) elicited a modest rise in both ^{45}Ca and ^{86}Rb outflow from islets perifused in the absence of glucose (data not shown).

Hydroxylamine (1 mM) provoked an immediate, sustained and rapidly reversible increase in ^{86}Rb fractional outflow rate from islets exposed throughout to 5.6 mM glucose (Fig. 3, upper panel). The capacity of hydroxylamine to stimulate ^{86}Rb fractional outflow rate was not impaired by the removal of extracellular Ca^{2+} (Fig. 3, upper panel). Thus, the magnitude of the increment in ^{86}Rb outflow evoked by hydroxylamine averaged 1.22 ± 0.14%/min in the presence and 1.44 ± 0.09%/min in the absence of extracellular Ca^{2+}, respectively ($P > 0.05$). By contrast, the addition to the perifusing medium of the hypoglycemic sulfonylurea glibenclamide (10 μM) coun-

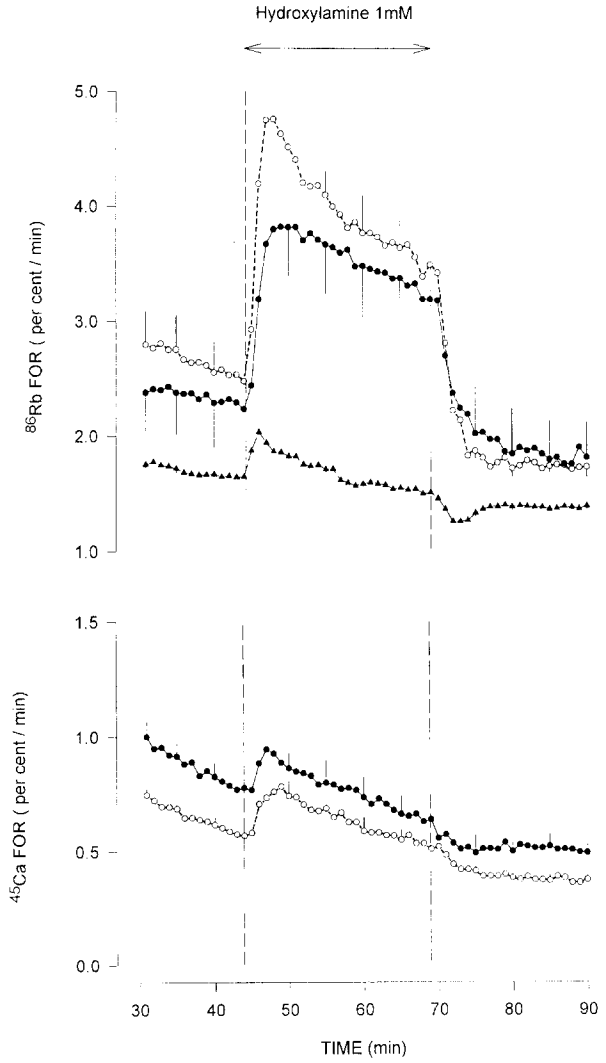

Fig. 3. Effect of hydroxylamine (1 mM) on ^{86}Rb (upper panel) and ^{45}Ca (lower panel) outflow from islets perifused throughout in the presence of 5.6 mM glucose. Basal media contained Ca^{2+} (●; 2.56 mM), were deprived of Ca^{2+} (○) or were deprived of Ca^{2+} and enriched with glibenclamide (▲; 10 μM). Mean values (±S.E.M.) refer to 4–5 individual experiments.

teracted the stimulatory effect of hydroxylamine (Fig. 3, upper panel). Whether in the presence or absence of extracellular Ca^{2+}, glibenclamide (1 or 10 μM) failed to completely abolish the hydroxylamine response (Fig. 3, upper panel and data not shown). Lastly, hydroxylamine slightly stimulated ^{45}Ca fractional outflow rate from islets exposed throughout to 5.6 mM glucose and perifused in the presence or absence of extracellular Ca^{2+} (Fig. 3, lower panel).

3.3. Effects of hydroxylamine on ^{86}Rb, ^{45}Ca outflow and insulin release from islets perifused in the presence of 16.7 mM glucose

In islets exposed throughout to 16.7 mM instead of 5.6 mM glucose, the addition of hydroxylamine (1 mM) provoked similar changes in ^{86}Rb fractional outflow rate (Fig. 4, upper panel). The drug induced a marked increase in ^{86}Rb outflow which persisted after extracellular Ca^{2+} omission. The presence of glibenclamide (10 μM) markedly inhibited, without completely suppressing, the stimulatory effect of hydroxylamine upon such a radioactive efflux. Fig. 4 (middle and lower panels) illustrates the effects of hydroxylamine (1 mM) on ^{45}Ca fractional outflow rate and insulin release from islets exposed throughout to 16.7 mM glucose and extracellular Ca^{2+}. Under such experimental conditions, the ^{45}Ca outflow and insulin

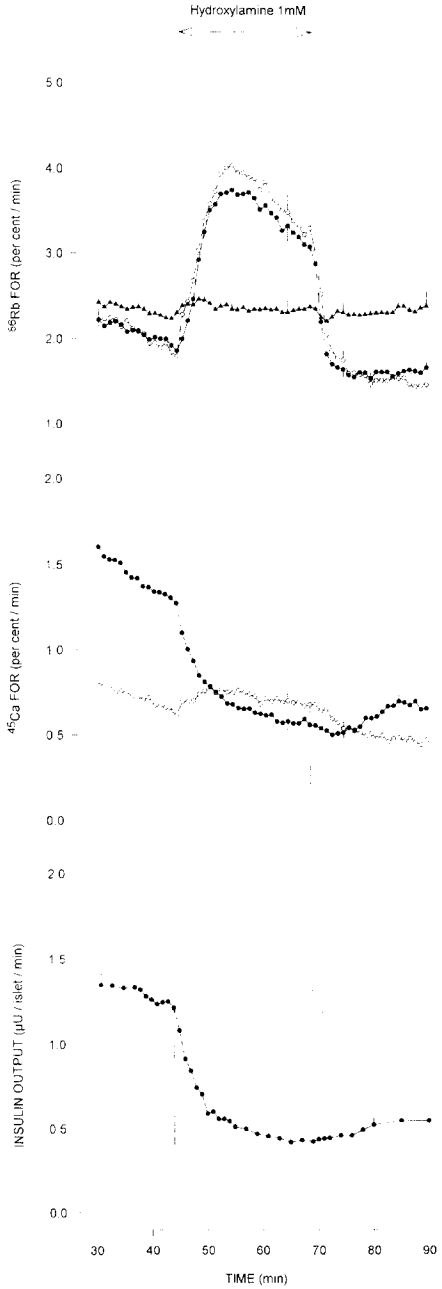

Fig. 4. Effect of hydroxylamine (1 mM) on ^{86}Rb (upper panel), ^{45}Ca outflow (middle panel) and insulin release (lower panel) from islets perifused throughout in the presence of 16.7 mM glucose. Basal media contained Ca^{2+} (●; 2.56 mM), Ca^{2+} and glibenclamide (▲; 10 μM) or were deprived of Ca^{2+} (0). Mean values (±S.E.M.) refer to 4–7 individual experiments.

secretory rates are marked and well sustained. Exposure to hydroxylamine provoked an immediate and pronounced inhibition of both ^{45}Ca fractional outflow rate and insulin output. In the presence of hydroxylamine, the ^{45}Ca fractional outflow rate (60–68th min) and the insulin output represented, respectively, 42.93 ± 3.44 ($n = 6$) and $36.13 \pm 3.45\%$ ($n = 4$) of the basal values recorded before the administration of the drug (40–44th min) ($P < 0.05$). The withdrawal of hydroxylamine from the physiological medium was followed by a slow reascension in both ^{45}Ca outflow and insulin release.

To further investigate the effects of hydroxylamine on ^{45}Ca movements and insulin release, the same experiments were conducted in the presence of 16.7 mM glucose but absence of extracellular Ca^{2+} (Fig. 4, middle and lower panels). In islets exposed to Ca^{2+}-depleted media, the rate of ^{45}Ca outflow before drug administration was lower than in the presence of extracellular Ca^{2+} ($P < 0.05$). The addition of hydroxylamine (1 mM) provoked a modest but sustained increase in ^{45}Ca fractional outflow rate. On removal of the drug, a reduction in ^{45}Ca outflow was recorded.

In another series of experiments, we characterized the effects of hydroxylamine on the glucose (16.7 mM)-induced biphasic changes in ^{45}Ca outflow. The latter modifications consisted of an initial and transient fall followed by a secondary rise in ^{45}Ca fractional outflow rate (Herchuelz et al., 1980; Lebrun et al., 1982a). When the perifusing medium contained hydroxylamine (1 mM) throughout, the addition of glucose still provoked a reduction in ^{45}Ca outflow but the secondary rise was completely abolished (data not shown).

3.4. Effects of hydroxylamine on K^+ depolarization-induced changes in ^{45}Ca outflow

Raising the extracellular concentration of K^+ from 5 to 50 mM provoked a rapid and sustained increase in ^{45}Ca outflow from islets perifused in the absence of glucose and presence of extracellular Ca^{2+} (data not shown). The presence of hydroxylamine (1 mM) in the basal medium failed to significantly affect this cationic response to K^+ (data not shown). Thus, the integrated outflow of ^{45}Ca measured during exposure to 50 mM K^+ averaged 1.03 ± 0.12 ($n = 5$) and $0.86 \pm 0.02\%$/min ($n = 5$) in the absence and presence of hydroxylamine, respectively ($P > 0.05$). Peak ^{45}Ca outflow observed during stimulation averaged 1.40 ± 0.14 ($n = 5$) and $1.26 \pm 0.02\%$/min ($n = 5$) in the absence and presence of hydroxylamine, respectively ($P > 0.05$).

3.5. Effects of hydroxylamine on the cytosolic free Ca^{2+} concentration

As previously reported (Antoine et al., 1993; Hellmann et al., 1992), measurements of fluorescence intensity from Fura-2 loaded islet cells indicated that an insulinotropic glucose concentration led to an increase in cytosolic calcium concentration ($[Ca^{2+}]_i$). In response to 16.7 mM glucose, single rat pancreatic islet cells responded with a brisk and sustained rise in $[Ca^{2+}]_i$ (Fig. 5). The subsequent addition of hydroxylamine (1 mM) reduced the increase in $[Ca^{2+}]_i$ brought about by 16.7 mM glucose.

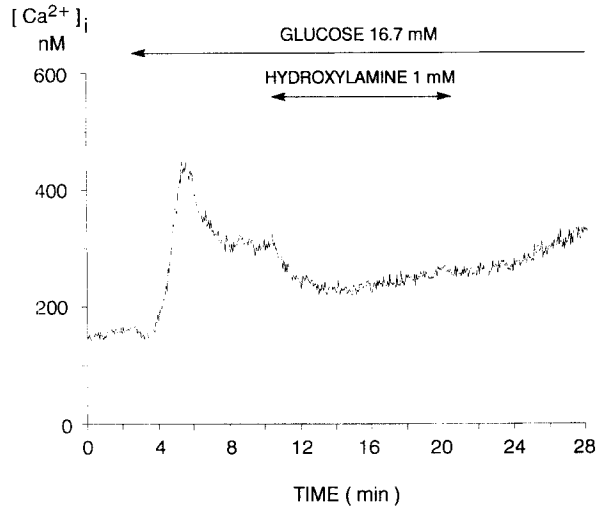

Fig. 5. Effect of hydroxylamine (1 mM) on glucose (16.7 mM)-induced increase in $[Ca^{2+}]_i$ in single pancreatic B-cells. Basal media contained glucose (2.8 mM) and Ca^{2+} (2.56 mM). Data are presented as mean signal of 19 different cells.

In the last series of experiments, we examined the effects of hydroxylamine on the KCl response. Depolarization with K^+ (50 mM) provoked a pronounced and sustained rise in $[Ca^{2+}]_i$ (data not shown). Under such experimental conditions, the further addition of hydroxylamine (1 mM) did not reduce the cytosolic free Ca^{2+} concentration (data not shown).

4. Discussion

Our data show that hydroxylamine provoked a concentration-dependent inhibition of the glucose-induced insulin release. High concentrations of the NO donor completely abolished the secretory response to the nutrient secretagogue. Moreover, addition of sodium nitroprusside or the NO donor 3-morpholinosydnonimine (SIN-1, unpublished observations) to rat pancreatic islets incubated in the presence of insulinotropic glucose concentrations also inhibited the insulin-releasing process. These findings confirm previous observations indicating that NO donors impaired the glucose-induced insulin secretory responses (Cunningham et al., 1994; Green et al., 1994).

The radioisotopic and microfluorimetric experiments performed in the present study support the contention that the hydroxylamine-induced decrease in insulin output may be attributable, at least in part, to changes in transmembrane ionic movements. Thus, the drug increased ^{86}Rb

outflow from pre-labelled and perifused rat pancreatic islets. Such findings can be interpreted as indirect evidence for an increase in membrane K^+ permeability (Lebrun et al., 1992; Malaisse et al., 1978; Pirotte et al., 1994). Activation of K^+ channels would cause membrane hyperpolarization leading to a reduction of voltage-gated Ca^{2+} channel activity, thus decreasing Ca^{2+} entry and ultimately inhibiting the secretory response. This cascade of events is substantiated by the observation that hydroxylamine inhibited ^{45}Ca outflow from islets exposed throughout to a medium containing 16.7 mM glucose and extracellular Ca^{2+}, a phenomenon representative of a decrease in $^{40}Ca^{2+}$ inflow into islet cells (Herchuelz et al., 1980; Lebrun et al., 1982a; Plasman et al., 1990). Moreover, the drug was also shown to abolish the increase in ^{45}Ca outflow brought about by a sudden rise in the extracellular glucose concentration, the latter increase reflecting a sustained stimulation of isotopic exchange between influent $^{40}Ca^{2+}$ and effluent ^{45}Ca (Herchuelz et al., 1980; Lebrun et al., 1982a; Plasman et al., 1990). Likewise, hydroxylamine counteracted the glucose-induced rise in cytosolic free Ca^{2+} concentration. Because the simultaneous measurement of insulin release from islets perifused in the presence of 16.7 mM glucose and extracellular Ca^{2+} displayed a time course parallel to that of the ^{45}Ca fractional outflow rate response, it can be suggested that the inhibitory effect of hydroxylamine on the insulin-releasing process results from a decrease of Ca^{2+} entry through voltage-sensitive Ca^{2+} channels.

The enhancing effect of hydroxylamine on ^{86}Rb fractional outflow rate as well as its lack of effect on the cationic responses to K^+ stimulation further indicate that the decrease of Ca^{2+} entry can be regarded as the consequence of K^+ channel activation. Indeed, the responses to high K^+ concentrations (≥ 50 mM) are known to be sensitive to Ca^{2+} entry blockers but are unaffected by the opening of K^+ channels (Lebrun et al., 1982a, 1992; Pirotte et al., 1994; Plasman et al., 1990). Incidentally, the present findings do not support the recent suggestion that NO could directly block the voltage-dependent Ca^{2+} channels (Krippeit-Drews et al., 1995).

Several findings argue in favour of a major role of ATP-sensitive K^+ channels in the cationic response to the NO donor. First, hydroxylamine increased ^{86}Rb outflow whatever the extracellular concentration of glucose (Henquin et al., 1992; Lebrun et al., 1992; Pirotte et al., 1994). Second, the acceleration of ^{86}Rb outflow persisted in the absence of extracellular Ca^{2+} (Lebrun et al., 1992; Pirotte et al., 1994). Third, the enhancing action of hydroxylamine on ^{86}Rb outflow was inhibited by glibenclamide, a hypoglycemic sulfonylurea reported to efficiently and selectively close the ATP-sensitive K^+ channels (Ashcroft and Rorsman, 1989; Lebrun et al., 1992; Malaisse and Lebrun, 1990).

It should be pointed out that the present findings do not bring information about the precise mechanism by which hydroxylamine activates the B-cell ATP-sensitive K^+ channels. However, impairment of glucose metabolism with subsequent changes in the ATP/ADP ratio (Tsuura et al., 1994), rather than a direct effect of NO on the channel activity, can be viewed as a determinant factor of the cationic response to the NO donor.

Glibenclamide severely reduced but failed to completely abolish the hydroxylamine-induced increase in ^{86}Rb fractional outflow rate. This modest but glibenclamide-resistant component of ^{86}Rb outflow could reflect the activation of a Ca^{2+}-sensitive modality of ^{86}Rb extrusion; as mediated by an intracellular redistribution of Ca^{2+} ions. This proposal is substantiated by the increase in ^{45}Ca outflow observed in prelabelled islets that were perifused in the absence of extracellular Ca^{2+} or in the presence of a non-insulinotropic glucose concentration (Lebrun et al., 1982b). A recent report showed that, in isolated rat pancreatic B-cells, aqueous NO induced intracellular Ca^{2+} mobilization with subsequent increase in the secretory activity (Willmott et al., 1995). Thus, it is tempting to speculate that the negative insulinotropic action of hydroxylamine, as mediated by the activation of ATP-sensitive K^+ channels, is partly blunted by a NO-induced intracellular Ca^{2+} redistribution.

The presence of glibenclamide in the incubation medium reduced the inhibitory effect of hydroxylamine and sodium nitroprusside on the release of insulin. Such a finding may be taken as further evidence that the negative insulinotropic action of NO donors is accounted for by their capacity to activate ATP-sensitive K^+ channels. Previous observations indicated that NO donors, such as sodium nitroprusside and *S*-nitroso-cysteine, enhanced the K^+ permeability of the B-cell membrane (Antoine et al., 1993; Krippeit-Drews et al., 1995; Tsuura et al., 1994).

Although glibenclamide attenuated the negative secretory action of hydroxylamine and sodium nitroprusside, the insulin output recorded in the simultaneous presence of the NO donors and the hypoglycemic sulfonylurea never reached the control value (16.7 mM glucose without added drug). Whatever the extracellular glibenclamide concentration (10^{-7} to 5.10^{-5} M), hydroxylamine and sodium nitroprusside still produced a $\approx 50\%$ reduction of the insulinotropic response to glucose. Such data imply that other mechanisms, unrelated to activation of ATP-sensitive K^+ channels, are involved in the NO donor modulation of the secretory process. Enzymatic alterations (Green et al., 1993, 1994), DNA damage (Green et al., 1994), or distal interference with the secretory sequence (Cunningham et al., 1994; Green et al., 1993) could be viewed as putative factors mediating the glibenclamide-resistant negative insulinotropic action of NO donors.

In conclusion, the present results confirm the capacity of NO donors to inhibit the glucose-induced insulin release. Our data also indicate that the negative insulinotropic action of hydroxylamine and sodium nitroprusside may result, at least in part, from the activation of ATP-sensitive K^+ channels. Opening of K^+ channels will

lead to a decrease in Ca^{2+} influx and reduction in $[Ca^{2+}]_i$, thereby uncoupling the stimulus-secretion sequence. Additional mechanisms, unrelated to changes in transmembrane ionic movements, could also be involved in the NO donors modulation of the insulin-releasing process.

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