Interaction between cAMP, volume-regulated anion channels and the Na^+-HCO_3^--cotransporter, NBCe1, in the regulation of nutrient- and hypotonicity-induced insulin release from isolated rat pancreatic islets and tumoral insulin-producing BRIN-BD11 cells

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Abstract. Soluble adenylyl cyclase (sAC) has been hypothesized to play a role in insulin secretion. The present study aimed to investigate the interaction between adenosine 3',5'-cyclic monophosphate (cAMP), volume-regulated anion channels (VRACs) and the electrogenic sodium bicarbonate (Na^+-HCO_3^-) cotransporter, NBCe1, in the regulation of nutrient- and hypotonicity-induced insulin release from rat pancreatic islets and tumoral insulin-producing BRIN-BD11 cells. In the islets, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) and 5-chloro-2-hydroxy-3-(thiophene-2-carbonyl) indole-1-carboxamide (tenidap) reduced glucose-stimulated insulin release, however, only NPPB suppressed the enhancing action of cAMP analogs upon such a release. Insulin output from the BRIN-BD11 cells was stimulated by 2-ketoisocaproate (KIC) or extracellular hypoosmolarity. cAMP analogs and 3-isobutyl-1-methylxanthine increased the insulin output recorded in the isotonic medium to a greater relative extent than that in the hypotonic medium. The secretory response to KIC or hypotonicity was inhibited by NPPB or tenidap, which both also opposed the enhancing action of cAMP analogs. Inhibitors of mitogen-activated protein (MAP) kinase decreased insulin output in isotonic and hypotonic media. The inhibitor of sAC, 2-hydroxyestriol, caused only a modest inhibition of insulin release, whether in the isotonic or hypotonic medium, even when tested at a concentration of 100 μM. The omission of $NaHCO_3$ markedly decreased the secretory response to KIC or extracellular hypotonicity. The omission of Na^+ suppressed the secretory response to extracellular hypotonicity. The observations of the present study do not support the hypothesis of a major role for sAC in the regulation of insulin release.

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Abbreviations: cAMP, adenosine 3',5'-cyclic monophosphate; 8-Br-cAMP, 8-bromo-cAMP; cAMP-AM, cAMP acetoxymethyl ester; db-cAMP, dibutyryl-cAMP; IBMX, 3-isobutyl-1-methylxanthine; KIC, 2-ketoisocaprate; MAP kinase, mitogen-activated protein kinase; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; Rp/Sp-8-Br-cAMPS, 8-bromoadenosine-3',5'-cyclic monosphosphorothioate, Rp/Sp isomer; VRAC, volume-regulated anion channel

Key words: adenosine 3',5'-cyclic monophosphate, volume-regulated anion channels, Na^+-HCO_3^- cotransporter NBCe1, insulin release, rat isolated pancreatic islets, BRIN-BD11 cells

Introduction

The expression of soluble adenylyl cyclase (sAC) was recently documented in insulin-secreting INS-1E cells by reverse transcription polymerase chain reaction, western blot analysis and immunocytochemistry (1). It was also hypothesized that an increase in the extracellular D-glucose concentration from 2.5 to 16.0 mM in INS-1E cells induces a delayed increase in levels of the secondary messenger, adenosine 3',5'-cyclic monophosphate (cAMP), mediated by bicarbonate, calcium and ATP-sensitive sAC, with subsequent phosphorylation of mitogen-activated protein (MAP) kinase. This hypothesis is not consistent with that formulated over 3 decades ago, stating that the glucose-induced increase in the cAMP content of insulin-producing cells is accounted for by the activation of membrane-associated adenylate cyclase by calcium-calmodulin (2).

Bicarbonate regulation of sAC was previously revealed to be physiologically relevant in sperm (3,4), bronchii (5) and the epididymis (6). In light of these observations, the possibility that a comparable mechanism exists in insulin-producing cells should be explored. Firstly, the knowledge that the omission of extracellular $NaHCO_3$ inhibits glucose-stimulated insulin release (7-9) was recently extended to the secretory response of rat isolated pancreatic islets to non-nutrient secretagogues (10). Secondly, the major fraction of CO_2 generated by the oxidative

catabolism of D-glucose in rat pancreatic islets is converted to HCO_3^- by the intervention of a mitochondrial type V carbonic anhydrase (11). Thirdly, it has been identified that the efflux of HCO_3^- from pancreatic islet cells may be mediated through volume-regulated anion channels (VRACs) gated in response to an increase in cell volume, itself provoked by a rise in D-glucose concentration or extracellular hypotonicity, at least following the initial 'phosphate flush' also attributable to the gating of VRAC (12). Finally, it was documented that insulin-producing cells express the electrogenic sodium bicarbonate (Na^+-HCO_3^-) cotransporter, NBCe1 (13). Inhibition of this cotransporter by 5-chloro-2-hydroxy-3-[thiophene-2-carbonyl]indole-1-carboxamide (tenidap) has been reported to result in alterations of $^{22}Na^+$ net uptake and insulin secretion in rat pancreatic islets (13,14). Therefore, NBCe1 may also play a role in the regulation of HCO_3^- fluxes in islet cells.

With this information in mind, the major aim of the present study, conducted in rat isolated pancreatic islets and tumoral insulin-producing cells of the BRIN-BD11 line (15), was to explore the effect of agents targeting carbonic anhydrase activity, VRAC, NBCe1 or MAP kinase activity, as well as the effect of changes in HCO_3^-, Cl^- and Na^+ extracellular concentration, upon the insulin secretory response to nutrient secretagogues (D-glucose, 2-ketoisocaproate [KIC]) or extracellular hypoosmolarity, as measured in the absence or presence of cAMP analogs and the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX).

Materials and methods

Drugs. Tenidap was a gift from Pfizer (Groton, CT, USA). All chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA) with the exception of IBMX, 1,4-diamino-2,3-dicyano-1,4-bis(*o*-aminophenylmercapto)butadiene ethanolate (U0126), 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one (PD98,059; Calbiochem, Merck Biosciences, Darmstadt, Germany), cAMP acetoxymethyl ester (cAMP-AM), adenosine-3',5'-cyclic monophosphorothioate (Sp-isomer) acetoxymethyl ester (Sp-cAMPS-AM), Rp-isomer of 8-bromo-cAMPS (Rp-8-Br-cAMPS), Sp-8-Br-cAMPS (Biolog, Bremen, Germany) and buffer compounds (Merck, Darmstadt, Germany). Tissue culture materials were purchased from Starstedt (Nümbrecht, Germany) and culture media from Invitrogen Life Technologies (Carlstadt, Germany).

Insulin secretion. The methods used to measure insulin secretion from the rat isolated pancreatic islets (16) or BRIN-BD11 cells (17) were performed as described previously.

Statistical analysis. All results are presented as mean ± SEM with the number of individual observations (n) or degrees of freedom (df). The statistical significance of differences between mean values was assessed using the Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

Experiments in rat isolated pancreatic islets. When incubated for 90 min, the insulin output of islets exposed to 8.3 mM D-glucose averaged 100.2±4.4 μU/islet (n=60), as compared with a basal value of 48.8±2.7 μU/islet (n=58) in the absence of hexose (P<0.001; Fig. 1). Glucose-stimulated insulin release was significantly increased by 8-Br-cAMP and dibutyryl-cAMP (db-cAMP; 1.0 mM each; P<0.04; Table I). The relative magnitude of the increase was not identified to be significantly different (P>0.22) between these cAMP analogs, with an overall mean value of 20.5±5.7% (df=115; P<0.001). As demonstrated in Fig. 1 (inset), the relative magnitude of the enhancing action of the cAMP analogs upon the secretory response to D-glucose was ~2-fold higher when corrected for basal values, with an overall mean enhancing action of 41.1±11.4% (df=115; P<0.001).

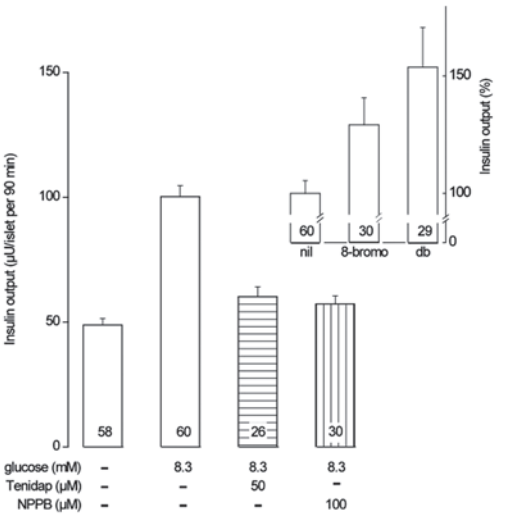

Figure 1. Insulin release by islets exposed to D-glucose, tenidap or NPPB. Inset presents the relative magnitude of the enhancing action of 8-bromo-cAMP (8-bromo) and dibutyryl-cAMP (db) on the insulinotropic action of D-glucose in the absence of any potential inhibitory agent, following correction for basal levels. Mean ± SEM values refer to the number of individual determinations presented at the bottom of each column.

Tenidap (50 μM) reduced the glucose-stimulated insulin release by 42.5±4.8% (df=54; P<0.001). Similarly, NPPB (100 μM) reduced the glucose-stimulated insulin release by 32.1±6.0% (df=58; P<0.001). The relative magnitudes of the reductions induced by tenidap and NPPB were not observed to be significantly different (df=112; P>0.18).

As demonstrated in Fig. 2, the relative extent of the inhibitory action of tenidap upon insulin release was not observed to differ significantly (P>0.78) in the presence (44.5±6.0%; df=26) or absence (42.2±6.0%; df=24) of 8-Br-cAMP, being significant (P<0.001) in both cases. The relative inhibitory action of tenidap upon insulin release did not differ significantly (P>0.52) in the presence (50.2±9.0%; df=26) or absence of db-cAMP (42.7±7.4%; df=28), and was significant (P<0.001) in both cases.

When compared within the same experiments, the relative extent of the inhibitory action of NPPB also failed to differ significantly (P>0.86) in the presence (27.1±8.1%; df=30) or absence (25.1±8.4%; df=29) of 8-Br-cAMP, which was significant (P<0.006) in both cases. In addition, the inhibitory action of NPPB was not identified to be significantly different (P>0.93) in the presence (36.2±9.2%; df=25) or absence (27.2±8.7%; df=27) of db-cAMP, and was significant (P<0.001) in both cases (Fig. 2).

Table I. Relative increment (%) of insulin release from islets incubated with D-glucose as provoked by cAMP analogs in the absence or presence of tenidap or NPPB.

cAMP analog	Inhibitor	Increment
8-Bromo-cAMP	-	+13.76±6.53[a] (df=59)
db-cAMP	-	+27.64±9.46[c] (df=56)
Both	-	+20.52±5.71[e] (df=115)
8-Bromo-cAMP	Tenidap	+28.49±8.39[d] (df=23)
db-cAMP	Tenidap	+14.23±12.26 (df=26)
Both	Tenidap	+20.94±7.59[b] (df=49)
8-Bromo-cAMP	NPPB	-7.32±5.63 (df=29)
db-cAMP	NPPB	+14.46±12.99 (df=26)
Both	NPPB	+3.00±6.92 (df=55)

[a]P<0.04; [b]P<0.009; [c]P<0.006; [d]P<0.004; [e]P<0.001. cAMP, adenosine 3',5'-cyclic monophosphate; db, dibutyryl; tenidap, 5-chloro-2-hydroxy-3-(thiophene-2-carbonyl)indole-1-carboxamide; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; df, degrees of freedom.

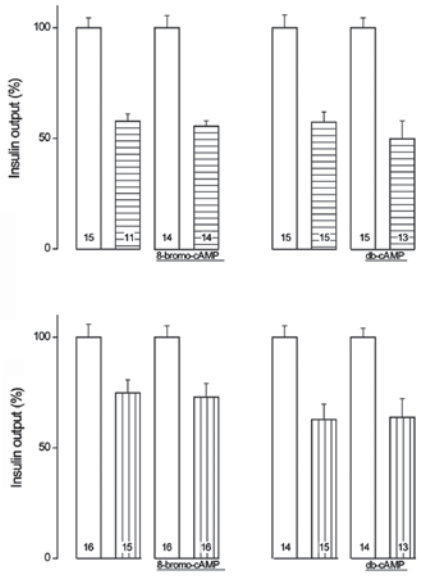

Figure 2. Inhibitory effect of tenidap (horizontally hatched columns; upper panel) and NPBB (vertically hatched columns; lower panel) on insulin release evoked by D-glucose in the absence or presence of 8-bromo-cAMP or db-cAMP in isolated pancreatic islets. Mean ± SEM values are expressed relative to the mean value found, within the same experiment(s), in the absence of any potential inhibitor of insulin secretion and refer to the number of individual observations indicated at the bottom of each column.

In the presence of tenidap, the two cAMP analogs augmented the mean levels of insulin release. The overall mean relative magnitude of this increase (20.9±7.6%; df=49; P<0.009) was almost identical to that recorded in the absence of inhibitors of insulin release (Table I). In the islets exposed to NPPB, however, the enhancing action of the cAMP analogs failed to achieve statistical significance, indicating that, under the present experimental conditions, NPPB suppressed an essential component of the secretory response to D-glucose.

Experiments in BRIN-BD11 cells

Reference data. Basal insulin output from BRIN-BD11 cells incubated in an isotonic medium (1.1 mM D-glucose) was 61.5±4.1 μU/ml/30 min (n=39). Output increased by 70.0±5.8 μU/ml/30 min (paired comparison within each experiment; n=33; P<0.001) when the BRIN-BD11 cells were exposed to a hypotonic medium [50 mM reduction in NaCl concentration (n=30) or a one-third dilution of the usual medium (n=3)]. No significant difference was observed (P>0.8) in insulin output under the latter two experimental conditions. Addition of KIC (10.0 mM) to the isotonic medium increased insulin output by 30.5±2.8 μU/ml/30 min (paired comparison; n=8; P<0.001).

Effect of cAMP analogs and phosphodiesterase inhibitors. cAMP-AM (0.1-0.2 mM) and IBMX (0.5 mM) increased the insulin output of BRIN-BD11 cells incubated in an isotonic medium to 254.8±15.1% (n=3; P<0.01) of the control value, compared with 139.3±12.8% (n=3; P<0.005) when BRIN-BD11 cells incubated in hypotonic medium were also exposed to cAMP-AM and IBMX. The hypotonicity-induced increment in insulin output, expressed as μU/ml/30 min, was ~2-fold lower (P<0.05) in the presence of cAMP-AM and IBMX (47.1±6.3; n=3) than in their absence (81.6±7.7; n=2). In addition, the insulin output of the BRIN-BD11 cells incubated in isotonic medium in the presence of cAMP-AM (0.1 mM) and IBMX (0.5 mM) was 201.8±15.4% (n=12; P<0.001) of the paired control value. The latter percentage was not noted to differ significantly (P>0.13) from that recorded in the first set of experiments.

Further experiments, all conducted in the isotonic medium, indicated that IBMX (0.5 mM) increased insulin output to 191.5±14.3% (n=12) of the paired value determined in its absence. When compared within the same experiments, the insulin output in the presence of IBMX (0.5 mM) was ≥97.3±3.4% (n=8) of that recorded in the presence of IBMX (0.5 mM) and cAMP-AM (0.1 mM).

Insulin output in the presence of other cAMP analogs in isotonic medium in the absence of IBMX was 105.4±4.4% (n=4) for 8-Br-cAMP (1.0 mM), 115.3±3.6% (n=4; P<0.025) for Sp-8-Br-cAMPS (0.1 mM) and 337.4±10.7% (n=4; P<0.001) for Sp-cAMPS-AM (0.1 mM).

For the purpose of comparison, the ratio for insulin output in the hypotonic/isotonic medium in the absence of any cAMP analog or phosphodiesterase inhibitor was 242.7±11.5% (n=16; P<0.001).

Effect of NPPB. The VRAC inhibitor, NPPB (0.1 mM), eliminated the secretory response to KIC (10 mM). Thus, instead of an increase in insulin output of 40.8±2.7% (n=2; P<0.05), as recorded in the presence of KIC but absence of NPPB, a mean decrease of 19.9±1.1% (n=2; P<0.05) was observed in the presence of KIC and NPPB. In the presence of NPPB, the cAMP analogs did not fully restore the insulinotropic action of KIC. Compared with a KIC-induced increment of 36.9±5.6 μU/ml/30 min, as observed in the absence of NPPB and a decrement of 17.9±2.6 μU/ml/30 min, as recorded in the presence of KIC and NPPB, values obtained in the presence of KIC and NPPB did not exceed +5.9±1.7 μU/ml/30 min in the presence of 1.0 mM 8-Br-cAMP, -2.5±0.4 μU/ml/30 min in the presence of 1.0 mM db-cAMP and -2.7±0.1 μU/ml/30 min in the presence of 1.0 mM dioctanoyl-cAMP or 0.1 mM

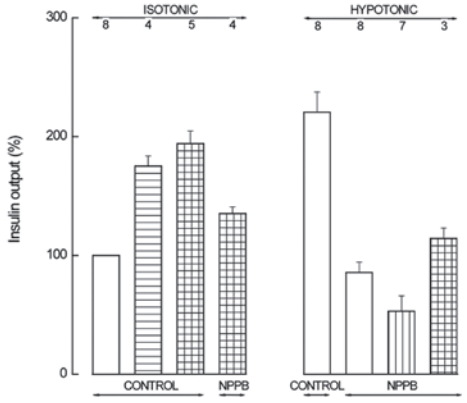

Figure 3. Insulin output from BRIN-BD11 cells incubated for 30 min in isotonic (left) or hypotonic (right) medium in the absence (control) or presence of NPPB. The incubation medium also contained IBMX (0.5 mM; horizontally hatched column) alone or together with cAMP-AM (0.1 mM; horizontally and vertically hatched columns) in the left panel; in the right panel, the incubation medium also contained a cAMP analog (0.1 mM 8-Br-cAMP, 1.0 mM db-cAMP, 1.0 mM dioctanoyl-cAMP or 0.1 mM 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester; vertically hatched column) or IBMX (0.5 mM) and a cAMP analog (0.1 mM cAMP-AM or 0.1 mM 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester; horizontally and vertically hatched column). Means ± SEM were collected in a series of 8 experiments, are expressed relative to the paired basal value in the isotonic medium and refer to the number of determinations indicated above each column. Reference basal value in the isotonic medium was 64.9±7.6 μU/ml/30 min (n=8). NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; IBMX, 3-isobutyl-1-methylxanthine; cAMP, adenosine 3',5'-cyclic monophosphate; AM, acetoxymethyl ester; db, dibutyryl.

2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester (n=2 in all cases). The paired difference from basal values obtained in the presence of KIC, NPBB and a cAMP analog yielded an overall mean value of +0.2±1.9 μU/ml/30 min (n=6), significantly higher (P<0.004) than the reduced insulin output recorded in the presence of KIC and NPPB and significantly lower (P<0.001) than the increased insulin output recorded in the presence of KIC.

NPPB (0.1 mM) also eliminated the secretory response evoked by extracellular hypotonicity. In a first series of 4 experiments, the output of insulin (μU/ml/30 min) was increased (P<0.01) from a basal value of 82.0±8.0 in an isotonic medium to 165.1±21.8 in the hypotonic medium. In the presence of NPPB, the release of insulin recorded in the hypotonic medium did not exceed 55.4±7.8 μU/ml/30 min (P<0.06 vs. basal value). Again, the cAMP analogs failed to restore the secretory response evoked by the exposure of the BRIN-BD11 cells to the hypotonic medium. Thus, in the presence of NPPB, the output of insulin (μU/ml/30 min) from cells exposed to the hypotonic medium averaged no more than 64.1±20.9 (n=3) in the presence of 1.0 mM 8-Br-cAMP, 24.5±5.7 (n=2) in the presence of 1.0 mM db-cAMP, 51.8 (n=1) in the presence of 1.0 mM dioctanoyl-cAMP and 28.3 (n=1) in the presence of 0.1 mM 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester. These observations indicate that the insulin output from the BRIN-BD11 cells exposed to hypotonic medium in the presence of NPPB and a cAMP analog was only 27.6±4.5% (n=7) of the value from cells exposed to hypotonic medium in the absence of NPPB.

Even in the presence of 0.5 mM IBMX, the release of insulin from BRIN-BD11 cells exposed to NPPB (0.1 mM) in the hypotonic medium did not exceed 51.3±4.8% (n=3) of the value determined in the hypotonic medium and absence of NPPB, despite the presence of 0.1 mM cAMP-AM (n=2) or 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester (n=1). However, the latter percentage was significantly higher (P<0.02) than that recorded in the absence of IBMX but presence of a cAMP analog (27.6±4.5%; n=7). The results demonstrate that the enhancing action of the phosphodiesterase inhibitor on insulin secretion remained operative in the cells exposed to NPPB.

These observations were confirmed in a further series of four experiments in which the release of insulin (μU/ml/30 min) from the BRIN-BD11 cells averaged 110.2±4.7 in the hypotonic medium, compared with (P<0.001) 47.8±3.6 in the isotonic medium and 48.1±3.5 in the hypotonic medium containing NPPB (0.1 mM). Fig. 3 presents the major observations relevant to analyses conducted in the presence of NPPB.

Effect of tenidap. In the absence of tenidap, which is postulated to be an inhibitor of the Na^+-HCO_3^--cotransporter, NBCe1, KIC (10.0 mM) increased insulin output by 25.7±5.8 μU/ml/30 min (n=3). The paired difference between insulin output in the presence of KIC and tenidap (50 μM) and the basal value in the absence of KIC yielded a mean negative value of -7.9±6.6 μU/ml/30 min (n=3). Tenidap decreased the KIC-stimulated insulin output to 61.3±2.0% (n=3; P<0.005) of its paired control value. In the presence of KIC and tenidap, 8-Br-cAMP or db-cAMP (both 1.0 mM) did not significantly increase insulin output whereby average output in the presence of the cAMP analogs was 109.8±7.4% (n=6) of the paired value recorded in their absence. Only dioctanoyl-cAMP (1.0 mM) and 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester (0.1 mM) augmented the insulin output evoked by KIC in the presence of tenidap from 53.0±11.5 to 97.5±11.6 μU/ml/30 min (n=3 in both cases; P<0.06). The latter mean value was not significantly different (P>0.65) from that recorded in the presence of KIC only (86.6±19.1 μU/ml/30 min; n=3).

In a series of eight experiments, the hypotonicity/isotonicity ratio for insulin release was 254.2±22.2% (n=8; P<0.001). When tenidap (50 μM) was present in the hypotonic medium, insulin release was decreased to 29.6±3.3% (n=8; P<0.001) of the paired value recorded in its absence and represented no more than 70.5±8.5% (n=8; P<0.01) of that recorded in the isotonic medium in the absence of tenidap. When 8-Br-cAMP (1.0 mM; n=4), db-cAMP (1.0 mM; n=3) or 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester (0.1 mM; n=1) was added to the hypotonic medium containing tenidap, the release of insulin was only 113.2±10.4% (n=8; p>0.25) of the paired value in their absence. Even in the presence of 0.5 mM IBMX and 0.1 mM cAMP-AM or 0.1 mM 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester, the output of insulin from the BRIN-BD11 cells incubated in the hypotonic medium containing tenidap did not exceed 127.6±19.2% (n=2; P>0.38) of the paired value in the hypotonic medium in the presence of tenidap only. In the presence of tenidap, only dioctanoyl-cAMP (1.0 mM) was observed to significantly increase the insulin output from the BRIN-BD11 cells exposed to the hypotonic medium from 33.3±7.8 to 101.1±15.0 μU/ml/30 min (n=2 in both cases; P<0.06). Even in BRIN-BD11 cells

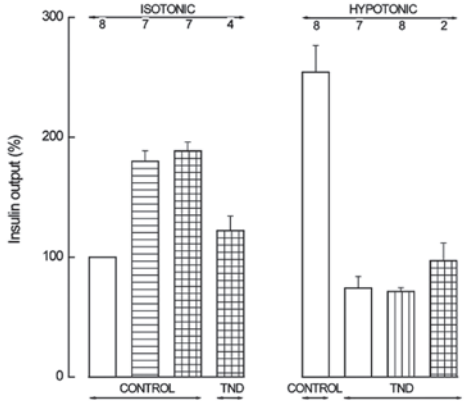

Figure 4. Insulin output from BRIN-BD11 cells incubated for 30 min in an isotonic (left) or hypotonic (right) medium in the absence (control) or presence (TND) of tenidap. The incubation medium also contained IBMX (0.5 mM; horizontally hatched column) alone or together with cAMP-AM (0.1 mM; horizontally and vertically hatched columns) in the left panel; in the right panel, the incubation medium contained a cAMP analog (1.0 mM 8-Br-cAMP, 1.0 mM db-cAMP or 0.1 mM 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester; vertically hatched column) or both IBMX (0.5 mM) and a cAMP analog (0.1 mM cAMP-AM or 0.1 mM 2'-O-monosuccinyladenosine-3',5'-cyclic monophosphate tyrosyl methyl ester; horizontally and vertically hatched column). Means ± SEM were collected in a series of 8 experiments, are expressed relative to the paired basal value in the isotonic medium and refer to the number of determinations indicated above each column. The reference basal value in the isotonic medium was 55.5±9.5 μU/ml/30 min (n=8; P>0.45, vs. the 8 experiments in Fig. 3). Control values of the hypotonic medium were 254.2±22.2% (n=8) of the paired basal value in the isotonic medium (P>0.25, vs. the 8 experiments in Fig. 3). Tenidap, 5-chloro-2-hydroxy-3-(thiophene-2-carbonyl)indole-1-carboxamide; IBMX, 3-isobutyl-1-methylxanthine; cAMP, adenosine 3',5'-cyclic monophosphate; AM, acetoxymethyl ester.

incubated in the isotonic medium in the presence of IBMX (0.5 mM) and cAMP-AM (0.1 mM), tenidap (50 μM) decreased insulin output from 86.9±7.0 μU/ml/30 min (n=4) recorded in the absence of tenidap to 54.3±5.8 μU/ml/30 min (n=4; P<0.02). The tenidap-induced decrease in insulin release was 32.6±4.8 μU/ml/30 min (n=4; P<0.008). Fig. 4 presents the major observations relevant to the analyses conducted in the presence of tenidap.

Effect of MAP kinase inhibitors. In the isotonic medium, the MAP kinase inhibitors, U0126 (10 μM) and PD98,059 (50 μM), reduced insulin output to 81.3±6.1% (n 4; P<0.06) of the paired control value. The relative extent of the reduction was not noted to be significantly different between the isotonic medium and either the isotonic medium containing 10 mM KIC or the hypotonic medium (df = 6; P>0.5).

Effect of 2-hydroxyestriol. The effects of the sAC inhibitor, 2-hydroxyestriol, upon the secretory response to extracellular hypotonicity were analyzed. Insulin output in the absence of 2-hydroxyestriol was 60.9±4.7 μU/ml/30 min (n=6; P<0.001) higher in the hypotonic medium than the isotonic medium. In addition, 2-hydroestriol (50 μM) was not observed to significantly affect insulin output in BRIN-BD11 cells incubated in isotonic or hypotonic medium. Insulin output in the presence of 2-hydroxyestriol (50 μM) was 94.0±8.2% (n=4; P>0.5) of the paired control value.

At a concentration of 100 μM, 2-hydroxyestriol exhibited a modest inhibitory effect, with an insulin output of 84.2±3.1% (n=8; P<0.002) of the paired control value. This reduction was not determined to differ significantly between the isotonic and hypotonic medium (P>0.36). Insulin release in BRIN-BD11 cells exposed to the hypotonic medium was inhibited by 2-hydroxyestriol (100 μM; P>0.5) or the membrane permeant, metabolically stable inhibitor of cAMP-dependent protein kinase, Rp-8-Br-cAMPS (100 μM), with an overall mean value of 82.8±5.6% (n=8; P<0.025) of the representative control.

Effect of HCO_3^- and/or Cl^- omission. The omission of $NaHCO_3$ severely decreased the secretory response to KIC (10 mM), the KIC-induced increment in insulin output not exceeding 5.0 μU/ml/30 min. Similarly, the paired ratio between insulin output in the hypotonic/isotonic medium was no more than 121.1±13.6% (n=3) in the absence of $NaHCO_3$, compared with 188.6±14.3% (n=12) in the presence of $NaHCO_3$ (P<0.05). In the absence of $NaHCO_3$, insulin output evoked by KIC or extracellular hypotonicity was decreased by 7.1±0.1 μU/ml/30 min (n=2; P<0.01) in the presence of 50 μM tenidap. In the presence of tenidap, neither 8-Br-cAMP (1.0 mM) nor db-cAMP (1.0 mM) were observed to significantly affect the insulin output of BRIN-BD11 cells exposed to KIC or extracellular hypotonicity in the absence of $NaHCO_3$, with a mean increment not exceeding 2.3±1.1 μU/ml/30 min (n=4). However, dioctanoyl-cAMP (1.0 mM), markedly increased insulin release from the BRIN-BD11 cells exposed, in the presence of tenidap and absence of $NaHCO_3$, to KIC or hypotonic medium. The increased insulin output attributable to dioctanoyl-cAMP was 63.9±1.6 μU/ml/30 min (n=2).

In the absence of Cl^- or Cl^- and HCO_3^- and in the absence or presence of the carbonic anhydrase inhibitor, ethoxyzolamide (6-ethoxy-2-benzothiazolsulfonamide; 0.5 mM), the paired ratio between insulin ouput in the hypotonic/isotonic medium was extremely low (P<0.04), averaging no more than 115.2±18.6% (n=3). Under these experimental conditions, the addition of 8-Br-cAMP (1.0 mM) to the incubation medium was not observed to significantly increase insulin output, which averaged 110.4±20.1% (n=3) of the paired control. Ethoxyzolamide (0.5 mM) increased insulin output from the BRIN-BD11 cells incubated in the absence of Cl^- and HCO_3^- to 187.2±4.1% (n=3; P<0.005) of the paired control value determined in the presence of Cl^- and HCO_3^- in the isotonic, hypotonic or hypotonic medium containing 8-Br-cAMP (1.0 mM).

Effect of Na^+ omission. Insulin release from BRIN-BD11 cells incubated in an isotonic medium deprived of Na^+ (substitution of NaCl (115 mM) by an equiosmolar mixture of 2-amino-2-hydroxymethyl-1,3-propanediol, N-methyl-D-glucosamine and sucrose and of $NaHCO_3$ (24 mM) by an equimolar amount of choline bicarbonate) was observed to be 2-3-fold higher than that found in the control isotonic medium. The hypotonicity/isotonicity ratio for insulin output, which exceeded 200% under the usual experimental conditions, did not exceed 103.6±3.3% (n=2; P>0.48) in the Na^+-free medium. When the BRIN-BD11 cells were exposed to the Na^+-free hypotonic medium, addition of 8-Br-cAMP (1.0 mM) or IBMX (0.5 mM) and cAMP-AM (0.1 mM) increased insulin output by 17.9 and 53.7%, respectively.

Discussion

The present study aimed to evaluate several novel observations associated with the interaction between cAMP, VRAC

and the Na^+-HCO_3^--cotransporter, NBCe1, in the regulation of nutrient- and hypotonicity-induced insulin release.

Studies in rat isolated pancreatic islets have demonstrated that tenidap and NPPB inhibit glucose-stimulated insulin release while 8-Br-cAMP and db-cAMP increase the insulinotropic action of hexose (12,13,18). cAMP analogs were observed to augment insulin output in the presence or absence of tenidap to the same extent and NPPB suppressed the enhancing action of the cAMP analogs on glucose-stimulated insulin secretion (Table I). The latter observation is consistent with the hypothesis that NPPB, by opposing the gating of VRACs, suppresses an essential component of the secretory response to D-glucose. It is well established that agents increasing the cAMP content of non-tumoral insulin-producing cells fail to augment insulin output from islets incubated at low D-glucose concentrations (18). By contrast, the maintenance of a marked positive response to the cAMP analogs in the presence of tenidap indicates that the role of NBCe1 in ionic fluxes does not represent an essential permissive process for the expression of D-glucose insulinotropic action.

In the analyses conducted in BRIN-BD11 cells, KIC was used as a nutrient secretagogue instead of D-glucose due to the poor secretory responsiveness of these tumoral insulin-producing cells to the hexose (19). The effects of cAMP analogs on insulin output from the BRIN-BD11 cells differed, in part, from those in isolated rat pancreatic islets. In contrast to the situation observed in rat islets (18), the phosphodiesterase inhibitor, theophylline, markedly enhanced insulin output from BRIN-BD11 cells incubated in isotonic medium even when the cells were exposed to a low D-glucose concentration (1.1 mM). In the absence of theophylline, Sp-cAMPS-AM (0.1 mM) and to a significantly more marked extent, Sp-8-Br-cAMPS (0.1 mM) also enhanced insulin output from the BRIN-BD11 cells incubated in isotonic medium.

In the BRIN-BD11 cells, the secretory response to KIC was eliminated in the presence of NPPB. Under these experimental conditions, the cAMP analogs simply brought insulin output to a level comparable to that obtained in the absence of KIC and NPPB. Similarly, in BRIN-BD11 cells exposed to the hypotonic medium in the presence of NPPB, the output of insulin recorded in the presence of cAMP analogs was not identified to be significantly different (df=9; $P<0.33$) from that recorded in their absence. Even in the presence of IBMX (0.5 mM) and a cAMP analog, insulin release from BRIN-BD11 cells exposed to hypotonic medium in the presence of NPPB (93.4±14.3 μU/ml/30 min; n=3) was not identified to be significantly different ($P>0.53$) from the corresponding basal value (81.8±10.6 μU/ml/30 min; n=3).

As demonstrated in rat isolated pancreatic islets, the effect of the NBCe1 inhibitor, tenidap, on insulin release from the BRIN-BD11 cells differed, in part, from that of NPPB. Tenidap was identified to decrease insulin output from BRIN-BD11 cells exposed to KIC or the hypotonic medium to mean values lower than the basal values recorded in the absence of tenidap. However, selected cAMP analogs restored insulin output from BRIN-BD11 cells exposed to KIC in the presence of tenidap to a level comparable to that recorded in the presence of KIC but absence of tenidap. Similarly, dioctanoyl-cAMP increased insulin output by ~3-fold in BRIN-BD11 cells exposed to hypotonic medium and tenidap.

A role for cAMP-responsive MAP kinase in the secretory activity of BRIN-BD11 cells was demonstrated by the inhibitory effects of U0126 and PD98,059 on basal and KIC- or hypotonicity-stimulated insulin release. Analyses conducted in the presence of 2-hydroxyestriol indicated a limited role for sAC in this secretory activity. It must be noted, however, that in both cases, basal and stimulated insulin output from the BRIN-BD11 cells was affected to a comparable extent by the tested inhibitors. However, this does not exclude a role for sAC in glucose-induced insulin release from isolated pancreatic islets.

Whilst the analyses conducted in BRIN-BD11 cells exposed to media deprived of HCO_3^- extend to the present experimental design the unfavourable effect of HCO_3^- omission on insulin secretion (7,8), the results revealed an unexpected enhancement of insulin secretion by ethoxyzolamide.

Finally, observations in BRIN-BD11 cells exposed to a Na^+-deprived medium indicate a favourable effect of Na^+ omission on basal insulin output which may be associated with a reduced consumption of ATP by Na^+,K^+-ATPase.

In conclusion, the present study demonstrates marked differences in the responsiveness to cAMP analogs depending on the conditions and agents used to stimulate or inhibit insulin secretion from rat isolated pancreatic islets or insulin-producing tumoral BRIN-BD11 cells. However, these observations do not appear to be to associated with the activation or inactivation of sAC. In addition, the inhibition of sAC (by 2-hydroxyestriol) or MAP kinase (by U0126 or PD98,059) did not have a significant effect on insulin output and insulin secretion recorded in the presence of KIC or in hypotonic medium, thus indicating that sAC and MAP kinase are not involved in the secretory response to a nutrient secretagogue or extracellular hypoosmolarity. In BRIN-BD11 cells, cAMP and its analogs consistently increased insulin output, whilst in isolated pancreatic islets these agents only amplified the secretory response to D-glucose. However, in both cases, VRACs appear to be involved in the effect of intracellular cAMP.

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