Glibenclamide but not other sulphonylureas stimulates release of neuropeptide Y from perifused rat islets and hamster insulinoma cells

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Abstract

We have studied the effects of first and second generation sulphonylureas on the release of insulin and neuropeptide tyrosine (NPY) from hamster insulinoma tumour (HIT-T15) cells and isolated rat islets. In the presence of 5·5 mmol/l glucose all sulphonylureas stimulated insulin release from the HIT cells (P<0·01 ANOVA, n ≥ 4) but only glibenclamide (GLIB, 10 µmol/l) stimulated the release of NPY (mean ± s.e.m. control 11·1 ± 1·3 vs GLIB 28·4 ± 4·1 fmol/h per 10⁶ cells, P<0·001, n=16). In isolated perfused rat islets both glibenclamide (10 µmol/l) (control 3·5 ± 0·3 vs GLIB 6·3 ± 0·2 fmol/min per islet, P<0·01, n=6) and tolbutamide (50 µmol/l) (control 4·7 ± 0·1 vs TOLB 6·7 ± 0·3 fmol/min per islet, P<0·01, n=6) enhanced glucose (8 mmol/l)-stimulated insulin release. However, only glibenclamide stimulated the release of NPY from the islets (control 3·4 ± 0·8 vs GLIB 24·5 ± 5 attomol/min per islet, P<0·01, n=6). Similar results were obtained in islets isolated from dexamethasone-treated rats. Glibenclamide treatment of HIT cells showed a prompt insulin release (10 min) while NPY secretion was slower (60 min), suggesting that internalization of the sulphonylurea is required to stimulate NPY release. Glibenclamide, the most common oral therapeutic agent in type 2 diabetes mellitus, is associated with release of the autocrine insulin secretion inhibitor, NPY.

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Introduction

Sulphonylureas enhance ß-cell insulin release by blocking the ATP-dependent K⁺ channel and are widely used in the treatment of type 2 diabetes mellitus. In chronic therapy the mechanism of action of sulphonylureas is less clear. Studies have shown that the long-term use of these oral hypoglycaemic agents does not increase basal insulin release or enhance insulin secretion in response to metabolic stimuli in patients with type 2 diabetes (DeFronzo et al. 1985). In experimental models, chronic treatment with sulphonylureas results in lower insulin content and reduced insulin responsiveness to metabolic stimuli (Duckwoth et al. 1972, Fineberg & Schneider 1980, Wajchenberg et al. 1980). One interpretation of the lower insulin content in this context is that it is secondary to excessive stimulation of ß-cell function. A study reporting the effect of chronic exposure of sulphonylureas on insulin secretion shows a reversible impairment of insulin release in isolated rat islets after a 24 h pre-exposure to glibenclamide (Gullo et al. 1991). Furthermore, desensitization of insulin release in rat islets has been demonstrated to occur secondarily to alterations in ionic fluxes in islets pre-exposed to glibenclamide but not tolbutamide (Rabuazzo et al. 1992). The mechanism underlying the ß-cell hyporesponsivity is not clear. However, among the sulphonylureas, glibenclamide is exceptional in accumulating progressively in ß-cells and pancreatic islets in mice (Hellman et al. 1984) and autoradiography using [³H]glibenclamide shows silver grains to be preferentially associated with ß-cells compared with non-ß endocrine cells (Carpentier et al. 1986). Thus it is possible that internalization of glibenclamide (Gorus et al. 1988), as well as causing a prolonged insulin secretory effect compared with tolbutamide, in some manner, contributes towards impaired insulin release following chronic exposure of islets to the drug.

Neuropeptide tyrosine (NPY), a 36-amino acid peptide, is widely distributed in the central and peripheral nervous systems and in the pancreas (Adrian et al. 1983, Gibson et al. 1984, Carlei et al. 1985). We and others have shown that NPY mRNA is present in rat and human islets and clonal ß-cell lines (Jamal et al. 1991, Wang et al. 1994, Bennet et al. 1996, Myrsen-Axcrona et al. 1997). Both...
in vivo and in vitro studies have demonstrated that NPY inhibits glucose-stimulated insulin secretion (Pettersson et al. 1987, Skoglund et al. 1991), probably acting via the Y1 receptor (Morgan et al. 1998). The inhibitory effect of NPY on insulin release in perfused islets isolated from both normal and dexamethasone-treated rats suggests it has an autocrine/paracrine role in the endocrine pancreas (Wang et al. 1994). In the same study, treatment of rat islets with NPY antisera increased insulin release and it was suggested that NPY is an endogenous tonal inhibitor of insulin (Wang et al. 1994).

In the present study, we have examined the effects of first and second generation sulphonylureas on their ability to stimulate insulin and NPY secretion in two different cell models. We have used rat islets that retain the physiological micro-architecture between the different islet cell types. In addition, to study the direct effects of the sulphonylureas on the β-cells we have used the predominantly insulin-secreting glucose-responsive hamster insulinoma cell line HIT-T15.

Materials and Methods

Chemicals

Glibenclamide, tolbutamide, chlorpropamide, dimethyl sulphoxide (DMSO), dexamethasone and all other chemicals and reagents, unless otherwise specified, were of Analar grade and obtained from Sigma Chemical Co. (Poole, Dorset, UK). Glipizide was a gift from Pfizer (Sandwich, Kent, UK) and gliclazide was obtained from Servier (Fulmer, Slough, UK).

Cell culture

HIT-T15 (HIT) cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were routinely cultured in RPMI 1640 medium (GIBCO-BRL, Paisley, UK) with 10%, vol/vol, foetal calf serum (FCS) supplemented with antibiotics as described earlier (Kulkarni et al. 1995a,b). All experiments were carried out between the passages 64 and 70. Plastic ware was obtained from NUNC (Roskilde, Denmark).

Experimental protocol

Glibenclamide, glipizide and gliclazide were dissolved in DMSO to a final concentration of 0·01% (vol/vol) and tolbutamide and chlorpropamide were dissolved in 50% ethanol (final concentration 1%, vol/vol) (Leclercq-Meyer et al. 1991). Experiments involved plating cells, at a density of 3 to 4 × 10⁵ cells/well, in 24-well plates in a total volume of 1 ml (Kulkarni et al. 1995a, Kulkarni et al. 1997). After reaching 70% confluence, cells were washed with glucose-free RPMI 1640 medium, and pre-incubated with serum-free and glucose-free medium for 2 h. Cells were then incubated in the absence or presence of different concentrations of sulphonylureas in medium containing 5·5 mmol/l glucose. Culture medium was collected, centrifuged at 500 g for 5 min to remove cell debris and the supernatant stored at −20 °C for subsequent RIA for insulin and NPY. Cell viability and cell counts were assessed at the end of each experiment by trypan blue exclusion using a haemocytometer.

Treatment of rats, islet isolation and perfusion

Male Wistar rats (250–300 g) were housed in the Biological Services Unit (ICSM, London, UK) and had free access to water and food. All experiments carried out on the animals complied with the regulations of the British Home Office. Two series of experiments were carried out. The first series involved the study of the effects of the sulphonylureas on insulin and NPY secretion in islets isolated from normal rats. In a subsequent series, the effects of the sulphonylureas were tested in islets isolated from a group of rats which had been injected subcutaneously with dexamethasone (4 mg/kg per day) for 10 days and the observed effects were compared with islets isolated from control animals injected with 0·9% saline. Rats were killed by CO₂ asphyxiation and islets were isolated by means of collagenase digestion using a modified method of Lacy and Kostianovsky as described earlier (Lacy & Kostianovsky 1967, Wang et al. 1994). Briefly, following isolation, islets were perifused with a 50:50 vol/vol mixture of Krebs Ringer buffer (KRB) and CMRL-1066 culture medium (GIBCO-BRL, Paisley, UK) supplemented with BSA (4 g/l), bacitracin (40 mg/g), ascorbic acid (80 mg/l), aprotonin (50 000 Kallikrein international units/l, TrasyloL, Bayer AG, Leverkusen, Germany) and glucose (2·8 mmol/l). The buffer perifusing the islets was gassed with O₂:CO₂, 9:5:5. The perfusion chambers were immersed in a water bath at 37 °C, and each chamber was perfused with buffer at 0·4 ml/min and fractions were collected for RIA.

RIA

Culture media were assayed in duplicate for insulin by an established RIA using porcine insulin standard and Glu5 antibody at a final dilution of 1:150 000 (Albano et al. 1972). The assay had a detection limit of 2 fmol/tube at 95% confidence limits with intra- and inter-assay coefficients of variation of 8·2 and 11·1% respectively. NPY in the culture media was assayed using antibody YN10 at a final dilution of 1:30 000 (Allen et al. 1984). Aliquots of perfusion effluent were rotary vacuum-dried (Savant Instruments, NY, USA) before analysis for NPY. The assay had a detection limit of 0·2 fmol/tube at 95% confidence limits and intra- and inter-assay coefficients of variation of 12·8 and 15·7% respectively. Potential
cross-reactivity of glibenclamide in the NPY assay was evaluated by performing the RIA in the presence and absence of the highest dose (10 µmol/l) of sulphonylurea used in the experiments and also at ten times this concentration.

Northern blot analysis and peptide content in cell extracts

HIT cells grown in 60 mm culture dishes were treated with the sulphonylureas for 12 h and harvested with 2.5 ml of guanidinium isothiocyanate solution. Samples were stored at −70 °C for subsequent extraction of RNA using the acid guanidinium isothiocyanate–phenol–chloroform procedure (Chomczynski & Sacchi 1987) or treated with acid ethanol overnight at 4 °C for estimation of peptide content (Kulkarni et al. 1995a). Poly(A)+ RNA was prepared from total RNA by oligo(dT12–18) cellulose chromatography (Sambrook et al. 1989). Filters were initially probed for NPY mRNA using a specific rat NPY cRNA probe, then stripped and re-probed for hamster insulin mRNA using a cDNA probe (Bretherton-Watt et al. 1989, Kulkarni et al. 1995a). Total RNA from rat hypothalamus was also probed for NPY and served as a reference for NPY mRNA (Jamal et al. 1991). Normalization of stripped filters was carried out by rehybridization with radiolabelled oligo(dT12–18) and the bands were quantified by an Autograph direct capture surface counter using Saxon Micro software as described earlier (Austin et al. 1994, Kulkarni et al. 1995a). Beta actin was used as control for loading (Austin et al. 1994). Results are expressed in arbitrary relative units of insulin or NPY mRNA.

Statistical methods

Cell and islet experiments were carried out on 6–16 different occasions in triplicate. Means of triplicate values were used to obtain an n=1. Results are expressed as means ± s.e.m. Experiments with gliclazide were carried out on four different occasions in triplicate due to a limited supply of the compound. Data was analysed by one-way ANOVA with subsequent post hoc Tukey tests. Values are considered significantly different if P<0.05.

Results

Clonal cells

Insulin release All sulphonylureas stimulated insulin secretion in a dose-dependent manner (Fig. 1A-D, Fig. 2A). The second generation sulphonylureas were more potent than tolbutamide and chlorpropamide (CHLOR) in stimulating insulin release. A maximal sevenfold increase was observed with 1 mmol/l of chlorpropamide (control 0.4 ± 0.1 vs CHLOR 2.9 ± 0.8 pmol/h per 10⁶ cells; P<0.01; n=6). The calculated EC₅₀ for glibenclamide was 76.9 ± 4.6 nmol/l with the observed maximal effect at 1 µmol/l. Glipizide and gliclazide stimulated insulin release with a threshold effect at a dose of 0.5 nmol/l. Cells treated with glipizide responded with a maximal threefold rise in insulin release at 10 nmol/l that did not significantly change with further addition of glipizide.

NPY release Among the sulphonylureas tested, only glibenclamide stimulated the release of NPY compared with untreated controls (Fig. 2B). There was a 1.4-fold increase in NPY at the dose of 0.5 µmol/l (control 11.1 ± 1.3 vs glibenclamide (GLIB) 16.1 ± 1.1 fmol/h per 10⁶ cells; P<0.01; n=16) and a 2.5-fold increase at 1 µmol/l (GLIB 27.3 ± 3.2 fmol/h per 10⁶ cells; P<0.001; n=16). There was no significant further increase in NPY at 10 µmol/l compared with 1 µmol/l (1 µmol/l NPY 28.4 ± 4.1 fmol/h per 10⁶ cells; P=NS; n=16). The EC₅₀ for NPY release was 181 ± 9 nmol/l. There was no significant increase in NPY release in response to any of the other sulphonylureas at the maximal tested concentrations (for concentrations see Fig. 1A-D) (control 11.1 ± 1.3; glipizide 9.1 ± 1.2; glibenclamide 10.8 ± 2.1; tolbutamide 12.1 ± 2.2; chlorpropamide 10.3 ± 0.8 fmol/h per 10⁶ cells; P=NS, control vs gliclazide or glipizide or tolbutamide or chlorpropamide, n=4–6).

Time-course kinetics of insulin and NPY release in response to glibenclamide To evaluate whether the insulin and NPY release in response to glibenclamide stimulation showed different kinetics, HIT cells were incubated with 10 µmol/l of the sulphonylurea for 10, 30, 60, 120 and 240 min. Figure 3A shows a significant increase in insulin release beginning at 10 min compared with control untreated cells (control 0.44 ± 0.05 vs GLIB 0.67 ± 0.05 pmol/10⁶ cells, P<0.02, n=4–6). However, NPY release was comparable to untreated cells up to 30 min and was significantly increased only at 60 min (control 20.1 ± 2.6 vs GLIB 51.8 ± 5.2 fmol/10⁶ cells, P<0.01, n=4–6, Fig. 3B). These data indicate that internalization of the sulphonylurea is likely to be necessary to promote NPY secretion. There was no cross-reactivity of the sulphonylurea in the NPY RIA at the concentrations used (or at 100 µmol/l) which excludes the possibility that the increase in NPY release in response to glibenclamide is secondary to an interference in the assay.

Effects of glibenclamide on insulin and NPY mRNA HIT cells incubated with glibenclamide (1 µmol/l) in the presence of glucose for 12 h did not show a significant increase in either insulin or NPY mRNA compared with untreated cells. The size of the NPY transcript in the HIT cells was confirmed by the presence of a band of similar size in the lane loaded with total RNA prepared from rat hypothalamus (Jamal et al. 1991) (Fig. 4, upper panel). The positive control (11 nmol/l glucose) showed a 1.5-fold
increase in insulin mRNA (control, 5.5 mmol/l glucose 100 ± 6% vs 11 mmol/l glucose 167 ± 10%, P<0.05, n=5) (Fig. 4, lower panel). In parallel experiments, measurement of peptide content in the glibenclamide-treated cells showed a significant reduction in insulin (control 11.21 ± 1.0 vs GLIB 9.37 ± 0.9 pmol/10^6 cells, P<0.05, n=3). By contrast, although a reduction was also evident in the NPY content this did not reach statistical significance (control 94.4 ± 8.8 vs GLIB 85.1 ± 7.2 fmol/10^6 cells, P=ns, n=3).

Isolated islets from normal rats
Following the observations in the HIT cells, two sulphonylureas – one first generation compound (tolbutamide) and one second generation compound (glibenclamide) – were examined for their effects on insulin and NPY release in perfused islets isolated from normal rats.

Effects on insulin release In all the islet experiments a significant increase in insulin secretion (68%, P<0.001) was observed when the glucose concentration was raised from 2.8 (time 0 to 40 min) to 8 mmol/l (time 40 to 80 min). Assessment of viability of islets at the end of each experiment showed a significant decrease in insulin release (time 161 to 200 min) when glucose was lowered to 2.8 mmol/l and a further significant elevation in insulin release (time 201 to 220 min) when glucose concentration was raised to 16 mmol/l.

During the treatment period (time 80 to 120 min), addition of glibenclamide at 10 µmol/l showed a further 80% increase in insulin secretion from treated islets compared with islets from untreated chambers (control 3.5 ± 0.3 vs GLIB 6.3 ± 0.2 fmol/min per islet; P<0.001, n=6) (Fig. 5A). Treatment with tolbutamide (50 µmol/l) showed a 63% increase in insulin release compared with islets from untreated chambers (control 4.1 ± 0.1 vs tolbutamide (TOLB) 6.7 ± 0.3 fmol/min per islet; P<0.01, n=6) (Fig. 5B).

Effects on NPY release The secretion pattern of NPY from perfused rat islets is shown in Fig. 6. The pattern of

Figure 1 Dose-dependent increase in insulin secretion in response to treatment with different sulphonylureas. HIT cells were treated with increasing concentrations of second generation (A) gliclazide (n=4), (B) glipizide (n=6), or first generation (C) tolbutamide (n=6) and (D) chlorpropamide (n=6) sulphonylureas for 120 min. *P<0.02, **P<0.01 vs control. Results are presented as means ± S.E.M.
NPY release in islets from normal rats, we therefore carried out the same experiment in islets from rats treated with dexamethasone. Pilot studies using tolbutamide (50 µmol/l) showed a significant increase in insulin release during the treatment period (control 6·4 ± 1·0 vs TOLB 9·8 ± 0·8 ± 8 fmol/min per islet; *P*<0·01, *n*=6), while no differences were observed in NPY release (control 10·8 ± 2·0 vs TOLB 11·2 ± 1·1 attomol/min per islet; *P*=NS, *n*=6).

**Effects on insulin release** Insulin secretion increased when glibenclamide (10 µmol/l) was added to the islets (time 81 to 120 min) (control 7·5 ± 0·1 vs GLIB 10·3 ± 0·3 fmol/min per islet, *P*<0·01, *n*=6) (Fig. 7A). During the 40 min immediately after treatment with glibenclamide (time 121 to 160 min), insulin secretion was significantly lower in the treated islets (control 7·3 ± 0·1 vs GLIB 6·3 ± 0·1 fmol/min per islet, *P*<0·01, *n*=6). No differences were observed during the subsequent periods of perfusion.

**Effects on NPY release** During glibenclamide treatment (time 81 to 120 min) an increase in NPY release was observed (control 12·1 ± 0·3 vs GLIB 22·3 ± 0·7 attomol/min per islet, *P*<0·01, *n*=6) (Fig. 7B), and continued to be elevated after glibenclamide was withdrawn, during the subsequent 40 min (time 121 to 160 min) (control 11·2 ± 0·5 vs GLIB 17·9 ± 0·5 attomol/min per islet, *P*<0·01, *n*=6). No significant differences were observed between treated and untreated chambers during the other periods.

**Discussion**

The present study shows a dose-dependent stimulatory effect of the second generation sulphonylurea, glibenclamide, on insulin and NPY release from clonal HIT cells. Glibenclamide also increased NPY secretion in islets isolated from normal and dexamethasone-treated rats. Treatment with glibenclamide did not significantly increase insulin or NPY mRNA in HIT cells. The other sulphonylureas tested – tolbutamide, chlorpropamide, gliclazide and glipizide – stimulated insulin release but not NPY release.

First generation sulphonylureas like tolbutamide have been largely replaced by second generation drugs like glibenclamide that are short-acting and more potent (Lebovitz & Feinglos 1983, Paice *et al.* 1985). Although the sulphonylureas continue to be used in the treatment of type 2 diabetes mellitus, long-term clinical studies have reported a secondary failure in increasing insulin secretion (Groop 1992). The cause of this ‘secondary-failure’ has been the subject of several investigations and have been ascribed to ‘β-cell exhaustion’ (Schauder *et al.* 1977, Gullo *et al.* 1991, Rabuazzo *et al.* 1992) and/or ‘β-cell...
desensitization’ to glucose (Hoenig et al. 1986, Bolaffi et al. 1988, Purrello et al. 1989) or other secretagogues (Grodsky 1989). An in vitro experiment (Gullo et al. 1991) has shown that 24 h exposure of islets to 100 nmol/l glibenclamide did not deplete the insulin content, suggesting that mechanisms other than insulin depletion are operative which contribute to the reduced insulin.

HIT cells express and secrete NPY-like immunoreactivity (Jamal et al. 1991). Further, NPY has been shown to be present in rat islets and to act as a paracrine factor inhibiting insulin secretion (Wang et al. 1994). Using immunocytochemistry and in situ hybridization, NPY has been localized to insulin-containing cells in rat islets (Waeber et al. 1993, Myrsen et al. 1995, Myrsen-Axcrona et al. 1997) and in addition has been shown to be localized to non-ß-cells including islet glucagon and islet somatostatin cells (Teitelman et al. 1993, Myrsen et al. 1995).

We observed an increase in NPY release associated with the insulin stimulatory effect, in HIT cells, only in response to glibenclamide but not to other sulphonylureas. This observation was confirmed using isolated perfused rat islets. Both tolbutamide and glibenclamide, however, increased insulin release in rat islets to levels comparable with those reported in other islet studies (Gullo et al. 1991, Rabuazzo et al. 1992). Our observation that among the sulphonylureas only glibenclamide is able to stimulate NPY release may be related to several possibilities. In HIT cells we observed a significant increase in insulin release at a threshold of 0·1 nmol/l of glibenclamide while a 100-fold higher concentration of the sulphonylurea was required to detect a significant increase in NPY release compared with controls. Earlier studies have reported that glibenclamide is exceptional among the sulphonylurea class of drugs to accumulate progressively in insulin-containing cells in rodent islets (Hellman et al. 1984). To test the possibility that accumulation of glibenclamide is a prerequisite to stimulate NPY release, we performed a

![Figure 3](image1.png)

**Figure 3** Glibenclamide stimulation of HIT cells shows different time-course kinetics of (A) insulin and (B) NPY release. HIT cells were treated with 10 μmol/l of glibenclamide and incubated for 10, 30, 60, 120 and 240 min as described in Materials and Methods. *P<0·02, **P<0·01 vs control. Results are presented as means ± S.E.M., n=4–6.

![Figure 4](image2.png)

**Figure 4** Glibenclamide does not alter insulin or NPY expression in HIT cells. Representative Northern blot (upper panel) showing the effect of glibenclamide (12 h treatment) on NPY and insulin mRNA using poly(A)+ RNA prepared from HIT cells. A significant increase in insulin mRNA was observed only in the lane with the positive control (Glu, 11 mmol/l glucose). The lower panel shows a quantitation of insulin mRNA expression in arbitrary units (mean ± S.E.M., n=5). *P<0·05, glucose vs control.
time-course experiment using HIT cells. While a prompt secretion of insulin was observed as early as 10 min of incubating the HIT cells with glibenclamide, a significant release of NPY was evident only at 60 min. The delayed release of NPY and the lack of cross-reactivity of the sulphonylurea in the NPY RIA supports the concept that this action of the sulphonylurea is likely linked to internalization. Further, glibenclamide has been shown to enhance insulin secretion largely by mobilizing stored granules, with newly synthesized hormone representing only a small proportion of this output (Schatz et al. 1975).

This observation is in agreement with our finding of the lack of an effect of glibenclamide on either insulin or NPY mRNA expression, indicating that the drug is probably effective only in the release of the stored peptides during this period (12 h). It is possible that longer periods of treatment and higher concentrations are required to alter the transcription of the insulin and NPY genes.

Secondly, in a recent report, Myrsen-Axcrona et al. (1997) proposed different pathways for NPY and insulin secretion in the clonal rat cell line RINm5F. In the context of treatment of β-cells with sulphonylureas, the presence of separate pathways for the two β-cell peptides may be related in part to the reported molecular heterogeneity and intracellular localization of the sulphonylurea receptor (Ozanne et al. 1995). Glibenclamide, in particular, has been shown to bind to a 140 kDa protein – different from the binding site occupied by other sulphonylureas such as glimepiride or other analogues containing a sulphonyl moiety (Kramer et al. 1994). In addition, sulphonylureas have been shown to stimulate exocytosis by directly activating components of the

Figure 5 Glibenclamide and tolbutamide stimulate insulin secretion from isolated rat islets in a perifusion system. Glucose concentration was 2·8 mmol/l from 0 to 40 min and 161 to 200 min, 8 mmol/l from 40 to 160 min, and 16 mmol/l from 161 to 200 min. The total treatment period was 40 min from 81 to 120 min. Filled bars represent control islets and hatched bars represent islets treated with (A) glibenclamide (10 μmol/l) or (B) tolbutamide (50 μmol/l) during 81–120 min. Glucose concentrations in the perifusion buffer are indicated on the right Y-axis (dashed line). *P<0·01, treated vs control. Results are presented as means±s.e.m., n=6.

Figure 6 Glibenclamide but not tolbutamide stimulates NPY release from isolated rat islets in a perifusion system. Glucose concentration and other details are as described in Fig. 5. Filled bars represent control islets and hatched bars represent islets treated with (A) glibenclamide (10 μmol/l) or (B) tolbutamide (50 μmol/l) during 81–120 min. Glucose concentrations in the perifusion buffer are indicated on the right Y-axis (dashed line). *P<0·01, treated vs control. Results are presented as means±s.e.m., n=6.
The presence, localization and characterization of these different mechanisms of action in the HIT cells may be useful to understand the secretory effects of glibenclamide affecting different β-cell peptides.

Finally, an earlier study has shown that GLP-1 (glucagon–like peptide–1) (7–36) amide stimulates insulin and NPY release from INS-1 insulinoma cells (Waer et al. 1993). The authors suggested that the incretin hormone may serve to autoregulate insulin release during periods of excessive secretion by stimulating a local inhibitor of insulin release such as NPY. Since dexamethasone treatment of rats is known to result in excess insulin secretion (Ogawa et al. 1992), we carried out experiments to assess the effects of glibenclamide stimulation on islets isolated from dexamethasone-treated rats. Glibenclamide induced a similar increase in NPY to that observed in islets from saline-treated rats. Interestingly, however, after withdrawal of glibenclamide, the NPY release continued to be high in the islets from the dexamethasone–treated group during which insulin release was observed to be significantly lower. Therefore, similar to the study in the INS-1 cells (Waer et al. 1993), it is possible that when insulin secretion is increased after treatment with dexamethasone, any additional stimulation such as glibenclamide treatment may lead to an autocrine/paracrine inhibitory mechanism resulting in the secretion of NPY which would restrain over-secretion of insulin. These findings along with the observation that NPY expression is induced by dexamethasone with a concurrent alteration in insulin release in rat islets and RINm5F cells (Wang et al. 1994, Myrson-Axcrna et al. 1997) collectively suggest that insulin and NPY secretion from the islets/β-cells is complex and may be regulated differently.

Type 2 diabetes patients unable to control hyperglycaemia on diet therapy alone are usually treated with oral hypoglycaemic agents, such as glibenclamide at a dose of 2·5 to 5 mg per day orally. These patients show serum levels ranging from 400 to 450 nmol/l four hours after ingestion of the drug (Grill et al. 1986, Ikegami et al. 1986). Assuming the concentration of the drug in the blood vessels supplying the pancreas and the islets is similar, this concentration would be in a similar molar range as that reported in this study required to stimulate insulin and NPY release.

Taken together the data in the present study shows an increase in insulin and NPY release following treatment with glibenclamide in HIT cells and islets isolated from normal and dexamethasone–treated rats. It is possible that the released NPY has an autocrine function to prevent excess secretion of insulin similar to the purported function of NPY secreted by GLP-1 stimulation in INS-1 cells (Waer et al. 1993). It is interesting that the other sulphonylureas did not stimulate NPY release and this may be related to the reported internalization of glibenclamide particularly to islet β-cells and/or to the heterogeneity of sulphonylurea receptors. Whether the action of glibenclamide in releasing NPY – an autocrine insulin inhibitory factor – contributes in any way towards the secondary failure associated with the use of the oral hypoglycaemic agent in type 2 diabetes requires further investigation.

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