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Evidence for hypothalamic K^+_{ATP} channels in the modulation of glucose homeostasis

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Abstract

Several lines of evidence support the hypothesis that ATP-sensitive K^+ channels (K^+_{ATP}) participate in the brain's regulation of peripheral glucose homeostasis. In testing this hypothesis we conducted a series of in vivo experiments using albino rats and bilateral intrahypothalamic injections of K^+_{ATP} channel blockers, glibenclamide and repaglinide. The results show that 0.2 and 2.0 nM injections of glibenclamide lowered blood glucose in a dose-dependent manner. During mild insulin-induced hypoglycemia, hypothalamic glibenclamide delayed recovery to normoglycemia. The impaired recovery was associated with a reduction in plasma norepinephrine ($P<0.001$), though circulating epinephrine and glucagon were not reduced. In a separate experiment, 2-deoxy-D-glucose (200 mg/kg) was intraperitoneally administered to produce neuroglucopenia. Hypothalamic injections of either glibenclamide or repaglinide significantly blunted compensatory hyperglycemic responses ($P<0.01$). In a feeding study, 2.0, but not 0.2 nM of hypothalamic glibenclamide, reduced chow intake over a 2-h period ($P<0.01$). The results support the hypothesis that hypothalamic K^+_{ATP} channels participate in central glucose-sensing and glucose regulation.

Keywords: Hypothalamus; Blood glucose; ATP-sensitive K^+ channel; Hypoglycemic; Glibenclamide; Repaglinide

1. Introduction

The physiologic relevance of ATP-sensitive K^+ channels (K^+_{ATP}) is best appreciated in pancreatic beta cells where the channels act as a link between the energy extracted from glucose catabolism and subsequent alterations in beta-cell membrane potential (Ashcroft et al., 1987). As such, the channels facilitate physiologically appropriate insulin release in response to elevated levels of glucose (see Nichols and Koster, 2002). Because the activity of the channels varies in proportion to the metabolism of glucose, the channels are suited to be active components in process of glucose-sensing. Several heteromeric forms of K^+_{ATP} channels have been identified in numerous extrapancreatic tissues, suggesting the possibility that the channels confer glucose sensing capability to a variety of organs.

The pancreatic form of the K^+_{ATP} channels is widely distributed throughout the central nervous system (Karschin et al., 1997) and several lines of evidence indicate functional relevance of the channels in neural tissue. Electrophysiologic studies have shown that modulation of the channels' activity causes alterations in the firing rates of sugar-sensitive neurons in the hypothalamus (Ashford et al., 1990; Lee et al., 1999) and in autonomic regulatory regions of the hindbrain (Dallaporta et al., 2000; Ferreira et al., 2001). Brain neurotransmitter release has been shown to be sensitive to glucose and K^+_{ATP} channel modulation (Amoroso et al., 1990). Additionally, studies have shown that portions of the signal transduction processes resulting from leptin and insulin receptor activation on hypothalmic neurons is mediated by K^+_{ATP} channels, and that these channel/receptor associations are absent in a rodent model of obesity and diabetes (Spanswick et al., 1997, 2000). Evidence of this type gives rise to the hypothesis that the physiological relevance of K^+_{ATP} channels in certain regions of the central nervous system

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is related to glucose-sensing in the service of energy balance, glucose counter-regulation and feeding behavior (Levin et al., 1999; Roane and Bounds, 1999; Yang et al., 1999; Mobbs et al., 2001; Schuit et al., 2001).

Few in vivo studies have addressed this hypothesis. However, a recent report utilizing a Kir6.2 knockout mouse supports the importance of central K^+_{ATP} channels in mediating glucose counter-regulatory responses (Miki et al., 2001). In this model, insulin injections produced an exaggerated hypoglycemia and delayed glycemic recovery. The Kir6.2$^{-/-}$ mice lack evidence of certain glucose detecting neurons in the hypothalamus. The animals' poor glucose compensatory capacity has been attributed to the absence of a sympathetically driven glucagon release, secondary to a defect in hypothalamic glucose-sensing.

In order to further explore the involvement of hypothalamic K^+_{ATP} channels in whole-animal glucose-sensing processes, we performed in vivo pharmacologic studies using two representatives of different classes of K^+_{ATP} channel blockers: the sulfonylurea, glibenclamide and the meglitinide compound, repaglinide. Previous studies have shown that glibenclamide and repaglinide affect insulin release by common and distinct processes, with the commonality occurring at K^+_{ATP} channels (Fuhlendorff et al., 1998) where the drugs are potent channel antagonists (Gromada et al., 1995). In the current study, these compounds were administered bilaterally into the mediobasal hypothalamic regions of albino rats and the subsequent blood glucose responses were recorded, before and after 2-deoxy-D-glucose-induced glucopenia. The effects of centrally administered glibenclamide on plasma epinephrine, norepinephrine and serum glucagon were also examined in rats during mild, insulin-induced hypoglycemia. Additionally, food intake was measured following hypothalamic glibenclamide. The results support glucose regulatory, and possibly appetitive roles for K^+_{ATP} channels in the medial basal hypothalamus, but in contrast to the Kir6.2$^{-/-}$ studies, the data do not indicate that the control of glucagon secretion is affected by channel activity in this particular region of the brain.

2. Methods

2.1. Animals

Male adult Sprague–Dawley rats (300–350 g) were obtained from the University of Louisiana at Monroe, College of Pharmacy vivarium and individually housed within a thermoregulated environment, with automatic control of a 12:12-h light/dark cycle. Rat chow and fresh tapwater were available ad libitum.

All animal protocols were approved the animal care and use committees at the University of Louisiana at Monroe and at Pennington Biomedical Research Center.

2.2. Surgical procedures

Rats were anesthetized by intraperitoneal injection of pentobarbital/atropine (50 and 1 mg/kg). The animals were placed into a stereotaxic apparatus, and fitted with bilateral 22-gauge stainless guide cannulae targeted towards the ventromedial nucleus of the hypothalamus. The stereotaxic coordinates for guide cannula placement, taken from the atlas of Paxinos and Watson (1986), were 2.8 mm posterior to bregma, 2.8 mm lateral to the midline, and 7.0 mm deep at a 13° angle toward the midline. The guide cannulae were secured with anchoring screws and dental acrylic. Injection cannulae were placed 9.8 mm deep to the dural surface. Post-experimental verification of cannula placement was assessed with the injection of dye (methylene blue in 50% glycerol). In all cases, the boundaries of the dye extended beyond the limits of the ventromedial nucleus and hence interpretations of the site of drug action can only be ascribed to the region of the medial basal hypothalamus. Poorly placed cannula extending into the caudal portions of the hypothalamus did not produce detectable drug effects and data from these animals are not included in the results. Immediately after the stereotaxic surgery, the animals were fitted with two vascular catheters (Silastic™ laboratory tubing, 0.025 in. ID × 0.047 in. OD). One catheter was placed into the right jugular vein and extended to the level immediately above the right atrium (approximately 3 cm deep), while the other catheter was placed into the right carotid artery and extended to the level of the aortic arch (approximately 4 cm deep). The catheters were filled with a 55% polyvinylpyrrolidone (PVP) solution containing heparin (50 U/ml), heat-sealed and remained sealed until the day of the study. The rats were housed in individual cages and allowed 7 days to recover following the surgery. All animals were gently handled on a daily basis until the day of the experiment to minimize possible confounding stress responses.

2.3. Experimental protocols

Rats were fully awake and freely moving in individual cages, and were food-deprived for 1 h before the start of the experiments, except for those animals in the feeding study (see below). All experiments were begun between 10:00 and 10:30 a.m. Each animal was given bilateral hypothalamic injections of vehicle (1% dimethyl sulfoxide (DMSO) in 25 mM $NaHCO_3$/NaCl buffer) or drug. At each site, a 400 nl injection was completed in approximately 1 min, and the injection cannula was left in place for an additional 1 min period. Both drugs, glibenclamide (Sigma/RBI, Natick, MA, USA) and repaglinide (a generous gift of Novo Nordisk Pharmacueticals), were administered as solutions in the above-mentioned vehicle. For evaluation of the effects of hypothalamic injections of glibenclamide on blood glucose levels, the control groups

received vehicle and the treated groups received doses of glibenclamide at 0.2 and 2 nmol/rat, half of each dose given on each side of the brain. Blood (0.1 ml) was withdrawn via the carotid artery catheter 20 min later. The sampling was continued, drawing once every 15 min for 2 h. For evaluation of the effect of hypothalamic injections of glibenclamide on glycemic recovery from insulin-induced hypoglycemia, the control group received vehicle and the treatment group received glibenclamide at a dose of 0.2 nmol/rat. Insulin (0.025 U/kg) followed by 0.1 ml of heparinized saline (5 U/ml) was administered through the venous catheter 20 min after brain injections; heparinized saline was used to fill dead volume in the catheter. Arterial blood was withdrawn immediately after intravenous injection. During the 2 h sampling period, blood was collected through the carotid artery catheter every 30 min to measure blood glucose, plasma catecholamines and glucagon. A three-way stopcock was used during blood withdrawal. Sample dilution by fluid in the dead space of the catheter and the syringe was avoided by withdrawal of ~ 0.3 ml of blood with the first syringe.A second syringe was used to collect blood samples (0.1 ml to measure blood glucose or 0.7 ml to measure hormones). The ~ 0.3 ml blood in the first syringe was administered back to the animals after blood samples were obtained followed by administration using a third syringe of 0.1 ml or 0.7 ml of saline. To evaluate the effects of hypothalamic injection of K^+_{ATP} channel blockers on blood glucose in 2-deoxy-D-glucose-treated animals, rats in the control group received vehicle, and those in the treatment group received glibenclamide or repaglinide, at a total dose of 0.2 nmol/rat. 2-Deoxy-D-glucose at a dose of 200 mg/kg was injected intraperitoneally 20 min after brain injections. The tip of the tail was cut using a surgical blade, and one drop of tail blood was taken for blood glucose measurements.

2.4. Blood glucose concentration measurement

Blood glucose concentration was quantitated with a OneTouch Ultra glucometer (Johnson and Johnson, Milpitas, CA). The calibration of this instrument was performed on artificially prepared rat blood containing defined concentrations of glucose between the ranges of 20 and 220 mg%. The resulting correlation between defined and measured blood glucose values was very high ($r^2 = 0.9989$).

2.5. Plasma sampling

Blood samples for catecholamine analysis were collected into heparinized 1.5 ml microcesntrifuge tubes (10 μl of 400 U/ml heparin) and centrifuged at $1000 \times g$ for 10 min at 4 °C. The plasma supernatant was aliquoted (340 μl) into microcentrifuge tubes with 20 μl 10 ng/ml internal standard, (dihydroxybenzylamine—DHBA), and frozen immediately at − 70 °C.

2.6. High-performance liquid chromatography (HPLC) determination of plasma catecholamines

On the day of measurement, samples were thawed at room temperature and the extraction of catecholamines was carried out according to the ESA plasma catecholamine methodology guide book. Briefly, plasma (280 μl) was mixed with 720 μl standard diluent, and the resulting solutions were added to alumina-containing extraction tubes. The alumina was washed twice and the catecholamines were eluted with 200 μl solution. A 50 μl portion of the eluate was injected into the chromatograph. Epinephrine and norepinephrine levels were determined by HPLC with electrochemical detection. The column employed was the ESA-HR 80 (80 × 4.6 mm, 3 mm particle size; ESA, Bedford, MA). The detector was a CoulochemTM model 5100 A (ESA) electrochemical detection system, with voltage potentials set to + 0.35 V for the guard cell, + 0.01 V for the first electrode and − 0.30 V for the second electrode. The assay was conducted at room temperature with an ESA Cat-A Phase buffer mobile phase and a flow rate of 1.5 ml/min. Primary standards were provided by ESA as stock solutions, all at concentrations of 1.0 mg/ml which were serially diluted to give final concentrations of 1 ng/ml. Typical retention times were 3.38 min for norepinephrine and 4.05 min for epinephrine. The limit of detection was approximately 20 pg/ml for both norepinephrine and epinephrine.

Plasma for glucagon was collected from arterial sample by similar methods as above, with the addition of 0.5 mM benzamidine HCl as a preservative in the collection tubes. Glucagon concentrations were determined using a radioimmunoassay kit from Linco Research (St. Charles, MO) where the sensitivity was ~ 25 pg/ml and the intra-assay coefficient of variation was approximately 4.5%.

2.7. Feeding experiment

The rats' average daily dark-period food intake was measured over three consecutive nights. Prior to the onset of the fourth dark cycle, food cups were filled with 50% of the amount of the chow consumed, on average, during the three previous nights. At 8:00 on the following morning animals were given hypothalamic injections of vehicle, 0.2 or 2.0 nM of glibenclamide. Twenty minutes after the injections, freshly filled food cups were returned to the animals and food consumption was recorded 30, 60 and 120 min later.

2.8. Control experiments

In order to control for the possibility of direct peripheral effects of arising from centrally administered drugs, one set of animals was given intravenous doses of glibenclamide identical in mass to the total doses injected into the brain, and blood glucose levels were measured over 2 h.

In order to control for the nonspecific effects of nonphysiologic substances injected into the hypothalamus,

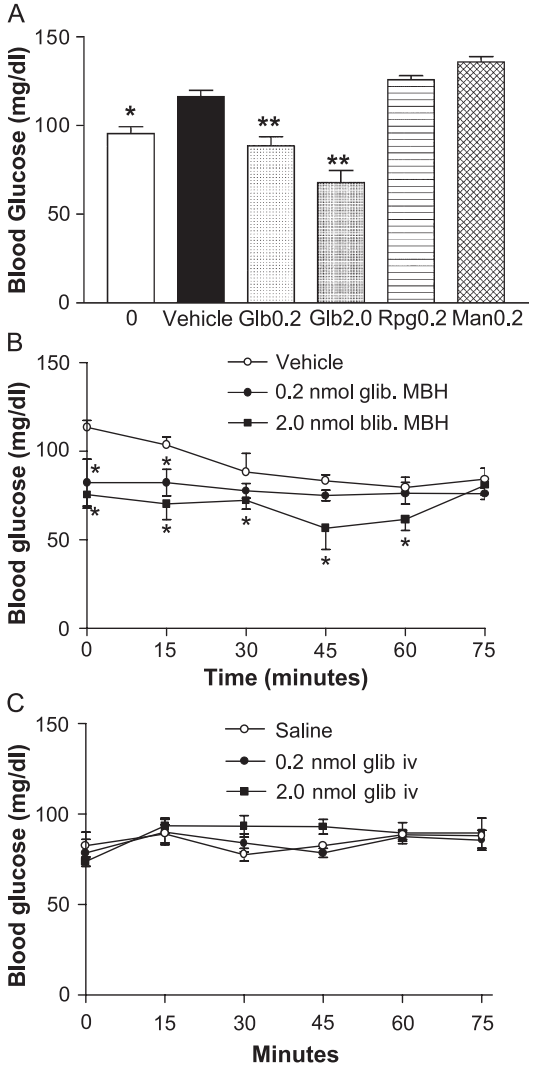

Fig. 1. The top panel, A, shows (from left to right) the blood glucose levels from uninjected sham control rats (0), and those receiving hypothalamic injections of vehicle, glibenclamide (Glb) 0.2 and 2.0 nmol, repaglinide (Rpg) 0.2 nmol or mannitol (Man) 0.2 nmol as an osmotic control. Measurements were made 20 min after the second bilateral hypothalamic injection. Comparisons between treatments were made by ANOVA with Dunnett's post hoc test where the vehicle-injected animals served as treatment group to which all other groups were compared. The data show that the blood glucose levels of the uninjected controls were lower (*$P<0.05$) than those of the vehicle-injected controls. Also, compared to the vehicle-treated animals, the hypothalamic administration of glibenclamide caused a lowering of blood glucose that varied with the dose of the drug (**$P<0.01$). Hypothalamic injections of repaglinide and mannitol caused slight, but statistically nonsignificant elevations of blood glucose. Panel B shows the effects of bilateral mediobasal hypothalamic (MBH) injections of 0.2 and 2.0 nM of glibenclamide (glib.) on blood glucose over a 75-min period beginning 20 min post-injection. Two-way ANOVA showed a statistically significant effect of due to the drug with the larger dose having the larger effect ($P<0.0001$). *Denotes $P<0.05$ for individual time points determined by Bonferroni-corrected *t*-test. Panel C shows the effects of the same doses of glibenclamide (glib.) used in panel B, but administered by intravenous injection rather than into the hypothalamus. Two-way ANOVA did not show statistically significant effects due to the drug. These data indicate that the hypoglycemic effects of glibenclamide, seen in panels A and B, are due to the drug's action in the central nervous system and are not caused by the drug escaping into the periphery following the hypothalamic injections.

animals were bilaterally injected with 0.2 nmol of mannitol and blood glucose was subsequently measured.

2.9. Statistical and data analysis

The statistical tests used were two-way analysis of variance (ANOVA) and one-way ANOVA followed by post hoc analysis with Dunnett's test. Groups were considered to be different from each other if $P<0.05$ ($n=3-10$ animals for all experiments). The area under the curves, shown in the inset of Fig. 2, were calculated by the trapezoidal rule. Independent baselines for each curve were set at the individual curve's *Y* value at time "0".

3. Results

The bilateral hypothalamic administration of the vehicle (1% dimethyl sulfoxide in 25 mM $NaHCO_3$/NaCl buffer) produced a mild, significant hyperglycemia compared to uninjected controls. Injections of 0.2 or 2.0 nmol of glibenclamide (half the dose given on each side) resulted in significant, dose-related decreases in blood glucose compared to vehicle-injected controls. Also, compared to the vehicle-injected controls, injections of repaglinide or mannitol did not significantly alter blood glucose. (Fig. 1A). Intrahypothalamic injections of 0.2 and 2.0 nM of glibenclamide produced dose-dependent effects of lowering glucose over the course of 75 min (Fig. 1B). Intravenous administration of the same quantities of glibenclamide did not cause statisti-

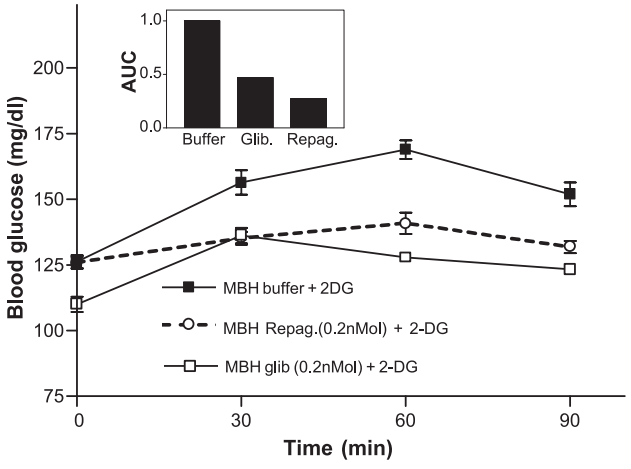

Fig. 2. Male albino rats were given 200 mg/kg 2-deoxy-D-glucose (2-DG) by i.p. injection at time 0. Twenty minutes prior, the animals had been given glibenclamide (glib), repaglinide (Repag) or vehicle bilaterally into the mediobasal hypothalamus (MBH). The increased blood glucose in response to 2-DG was significantly reduced by either K^+_{ATP} channel blocker (independent two-way ANOVA, $P<0.001$, $n=6$ per treatment). The inset figure shows, from left to right, the relative areas under the respective curves (AUC) for buffer-, glibenclamide- and repaglinide-treated animals. The blood glucose values at "0" time point for each independent treatment served as baseline for each of the corresponding curves.

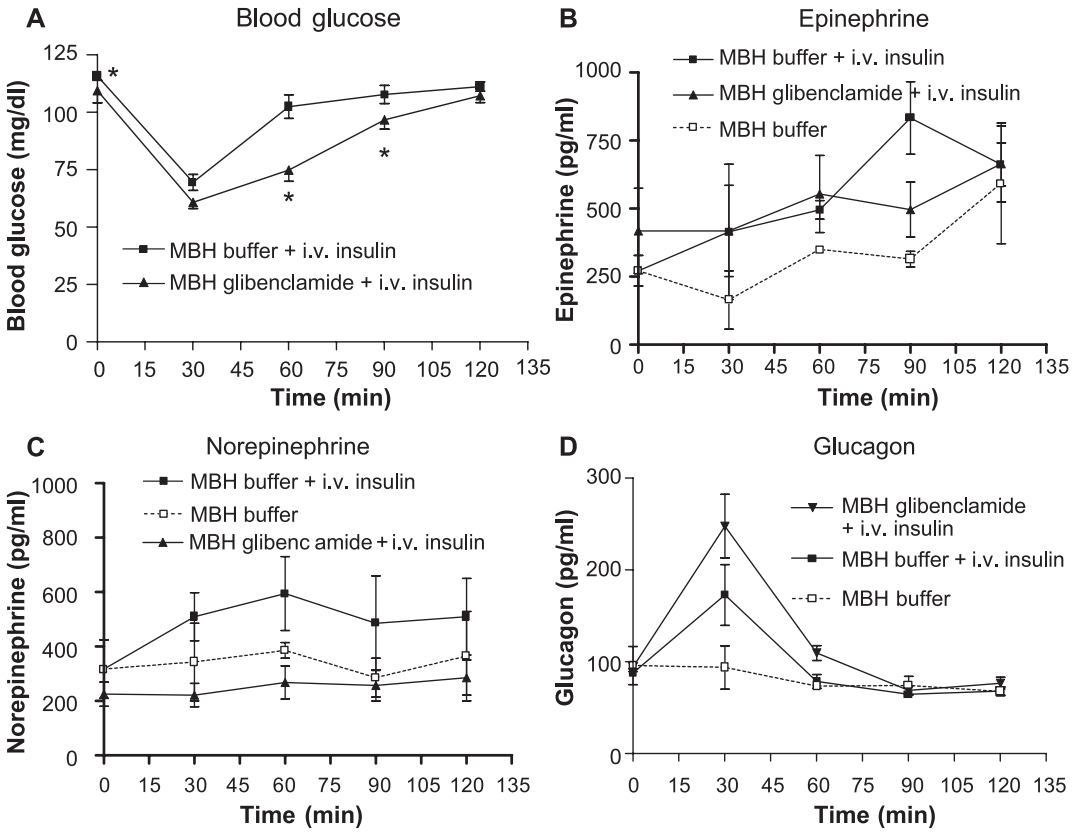

Fig. 3. Animals ($n=4-10$ per group) received bilateral mediobasal hypothalamic (MBH) injections of 0.2 nM of glibenclamide or vehicle 20 min prior to time "0". At time "0" the animals were given 0.025 U of insulin or saline, i.v. Glibenclamide significantly retarded recovery from mild hypoglycemia (A, $P=0.007$). No significant effect of the drug was seen on epinephrine responses (B). Plasma norepinephrine (C) was markedly reduced by glibenclamide compared to hypoglycemic animals receiving vehicle ($P=0.0006$). Glucagon responses (D) were higher in glibenclamide-treated animals compared to insulin-treated, hypoglycemia control ($P<0.05$). *Denotes $P<0.05$ for individual time points determined by Bonferroni-corrected t-test.

cally significant changes in blood glucose over the same time period (Fig. 1C).

In the neuroglucopenia study, both glibenclamide and repaglinide (0.2 nM) markedly reduced the hyperglycemic response to peripherally administered 2-deoxy-D-glucose (Fig. 2).

The recovery from mild, insulin-induced hypoglycemia was obtunded in rats receiving glibenclamide into the mediobasal hypothalamus (Fig. 3A). Blood samples taken during the course of this experiment showed that hypoglycemia produced equivalent elevations in plasma epinephrine in both glibenclamide- and vehicle-injected animals (Fig. 3B). In contrast, however, plasma norepinephrine responses were notably blunted during hypoglycemia in glibenclamide-treated animals (Fig. 3C) with the levels of norepinephrine being below normoglycemic controls at all time points. Serum glucagon levels in glibenclamide-treated animals were significantly elevated above hypoglycemic, vehicle-injected controls (Fig. 3D).

In the feeding experiment, only the higher dose of 2.0 nM reduced food consumption in 50% overnight-restricted animals. The lower dose (0.2 nM), which was used in all

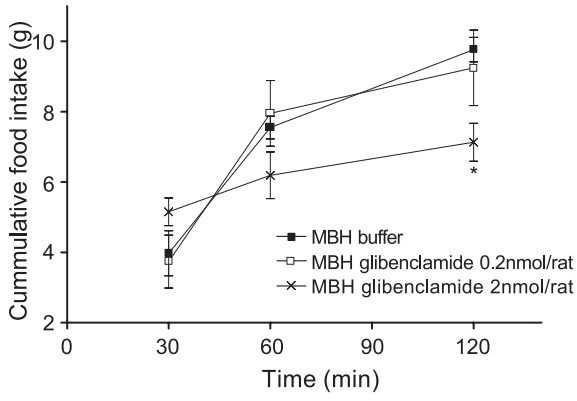

Fig. 4. Rats were food restricted by 50% of their normal food intake, overnight and were given bilateral injections of 0.2 or 2.0 nmol glibenclamide into the mediobasal hypothalamus (MBH) the following morning. Twenty minutes after the injections, chow was returned to the cages and consumption was recorded. Animals receiving the larger doses of glibenclamide showed a significant reduction in cumulative food intake over 2 h of re-feeding ($P<0.01$, $n=6$/group). *Denotes $P<0.05$ for individual time points determined by Bonferroni-corrected t-test.

other experiments for effects on blood glucose, had no discernable effect on chow intake (Fig. 4).

4. Discussion

A summary of the current results shows that bilateral injections of small quantities of the K^+_{ATP} channel blocker, glibenclamide, into the mediobasal hypothalamus lowered blood glucose when compared to vehicle-injected controls and impaired recovery from mild, insulin-mediated hypoglycemia in a manner consistent with previous studies (Zhang and Roane, 2001). These results are in agreement with the hypothesis that K^+_{ATP} channels in the mediobasal hypothalamus participate in central glucose-sensing mechanisms that serves glucose counter-regulation. The peripheral mechanism by which this happens is uncertain, but does not appear to directly involve the release of glucagon or epinephrine. Hypothalamic injections of either glibenclamide or repaglinide interfered with the hyperglycemic response to 2-deoxy-D-glucose-induced glucoprivation, indicating that K^+_{ATP} channels are important to the maintenance of normal glucose homeostasis. The same dose of glibenclamide that significantly altered glucose homeostasis did not affected food intake, though a 10-fold higher dose reduced feeding in food-restricted animals. We interpret this latter finding as a possible indication that the control of appetitive behavior, compared to glucose homeostasis, is less easily affected by processes involving of K^+_{ATP} channels in the mediobasal hypothalamus. Similar findings of differing sensitivities of glucose homeostasis and food intake to pharmacologic effects mediated through the hypothalamus have been previously reported. (Obici et al., 2001). However, we cannot rule out the possibility that a food-intake experiment employing milder conditions of food restriction (<50%) may show greater sensitivity to the feeding effects of lower doses of glibenclamide. Previous reports on the feeding effects of centrally administered sulfonylureas have been variable (Roane and Boyd, 1993; Bounds et al., 1999).

The data in Fig. 1 show that the injection of the vehicle alone or vehicle containing mannitol into the mediobasal hypothalamus raised blood glucose levels, compared to uninjected controls. This finding is not surprising because the vehicle did not contain glucose, and mannitol does not provide a ready source of metabolizable energy. The injections likely caused a dilution of local extracellular glucose concentrations in this glucose-sensing region of the brain and, as such, initiated a mild compensatory response seen as an elevation in blood glucose. Previous studies in rats have shown that localized glucopenia within the mediobasal hypothalamus can cause glucose counter-regulatory responses (Borg et al., 1995) and infusions of glucose-containing buffer into the ventromedial hypothalamus can prevent peripheral compensatory responses to systemic hypoglycemia (Borg et al., 1997). Our interpretation is consistent with these reports.

The data in Fig. 1 demonstrate the notable finding that hypothalamic injections of glibenclamide lowered blood glucose in a dose-related manner, and repaglinide had no effect, relative to vehicle-injected controls. This finding of a lack of effect of repaglinide was also evident at the "0" time point in Fig. 2, which reflects the blood glucose status of the animals immediately prior to the administration of 2-DG. However, following the injections of 2-deoxy-D-glucose, the magnitude of the effect of repaglinide on blood glucose exceeded that of equimolar injections of glibenclamide (inset of Fig. 2). The findings are consistent with the results of previous comparative studies of the potencies of glibenclamide and repaglinide. In cell-based assays of insulin release, repaglinide showed diminished functional effects as glucose concentrations approach 0 mM, while glibenclamide effects were not reduced in the absence of glucose. However, as glucose levels were raised, repaglinide exhibited greater potency than glibenclamide, thus leading to the conclusion that replaginide's potency varies from lesser than to greater than glibenclamide, depending on ambient glucose concentrations (Fuhlendorff et al., 1998). Extracellular brain glucose concentrations have been estimated to be 0.3 to 1.3 mM (Silver and Erecinska, 1994; Jacob et al., 2002). It could be the case that during normoglycemia or hypoglycemia, brain levels of extracellular glucose are sufficiently low such that the effects of repaglinide are negligible, but when glucose availability increases, as seen following the administration of 2-deoxy-D-glucose, repaglinide effects are pronounced and exceed those of glibenclamide.

It is worth noting that glibenclamide and repaglinide are both used as insulin secretagogues where their primary mechanism of action has been attributed to antagonism of K^+_{ATP} channels on cell surface membranes. Both compounds lead to decreased K^+ conductance, which promotes membrane depolarization, increased Ca^{2+} conductance, and subsequently, Ca^{2+}-mediated insulin release. It is presumed that by virtue of this action, glibenclamide and repaglinide would exert similar effects on neuronal tissue to facilitate transmitter release (Amoroso et al., 1990). However, glibenclamide has been identified as having intracellular activities which promote neurotransmitter release apart from it actions as a K^+_{ATP} channel blocker (Renstrom et al., 2002). Glibenclamide may stimulate vesicular release through direct interaction with exocytotic machinery in a PKC-dependent mechanism (Eliasson et al., 1996), and by activating SUR proteins linked to Cl^- channels on secretory vesicles (Barg et al., 1999). These intracellular phenomena do not appear to be relevant to the actions of repaglinide, (Fuhlendorff et al., 1998; Gromada et al., 2002). In the current study, both drugs affect glucose homeostasis in a manner consistent with the interpretation that the effects are mediated through K^+_{ATP} channels.

Our findings are in agreement with numerous previous reports which have identified the ventromedial region of the hypothalamus as an important in central glucose-sensing and in the initiation of counter-regulatory responses to hypoglycemia. In vivo rat studies have shown that ablative lesions

of the ventromedial nuclei block the peripheral release of epinephrine, norepinephrine and glucagon during hypoglycemia (Borg et al., 1994). Additionally, localized infusion of 2-deoxy-D-glucose into the ventromedial area elicits the release of each of these peripheral compensatory factors in normoglycemic animals (Borg et al., 1995), while in hypoglycemic animals, the application of glucose into the ventromedial hypothalamus inhibits counter-regulatory hormone release (Borg et al., 1997). Supporting evidence for K^+_{ATP} channels involvement in these processes are seen in neuroanatomical studies which show that nuclei within the medialbasal hypothalamic region contain relatively high densities of glucose responsive neurons (Funahashi et al., 1999), and expression of K^+_{ATP} channel subunits occurs within this region (Dunn-Meynell et al., 1998). Many sugar-sensitive neurons in the mediobasal hypothalamus show in vitro sensitivity to sulfonylureas (Ashford et al., 1990). K^+_{ATP} channel activity in hypothalamic neurons has been proposed to be important to the regulation of the activities of neurons expressing leptin receptors (Spanswick et al., 1997) and insulin receptors (Spanswick et al., 2000). These latter findings become relevant to the current study in light of reports showing that the central administration of either leptin (Lin et al., 2002) or insulin (Obici et al., 2002) alters peripheral glucose homeostasis. Additionally, recent studies have demonstrated that proopiomealnocortin (POMC) neurons in the lower medial hypothalamus express Kir6.2/SUR1 and are glucose- and tolbutamide-responsive, i.e., the neuronal firing rates are increased by glucose or K^+_{ATP} channel-closing sulfonylureas (Ibrahim et al., 2003). Presumably, an increased firing rate of glucose-sensitive POMC neurons leads to an increase in the release of alpha melanocyte-stimulating hormone (α-MSH) and the subsequent activation of central melanocortin receptors which ultimately affect factors such as increased in peripheral glucose uptake and diminished hepatic glucose output (Obici et al., 2001), and leptin-regulated insulin release (Muzumdar et al., 2003). Such findings are consistent with the data presented in the current manuscript and suggest a modulating role or "gain-setting" role for K^+_{ATP} channels in determining the sensitivity of hypothalamic glucose regulating pathways.

Other important evidence supporting a role for K^+_{ATP} channel in central glucose sensing and counter-regulation comes from studies in Kir6.2 knockout mice (Miki et al., 2001). Kir6.2$^{-/-}$ mice show a number of aberrations in compensatory responses to hypoglycemia, most of which are consistent with the results seen in the current study. The similarities include the findings that both models show blunted responses to 2-deoxy-D-glucose, both show impaired recovery from insulin-induced hypoglycemia, and neither model indicated an impairment of epinephrine release during hypoglycemia. In contrast, however, Kir 6.2$^{-/-}$ show an absent glucagon response during hypoglycemia, while our drug model shows a robust glucagon response with no measurable norepinephrine response. Norepinephrine responses to hypoglycemia have not been reported for Kir6.2$^{-/-}$ mice. The currently available information does not allow us to fully reconcile this difference, though our data supports the contention that glucagon release is not predominantly mediated by glucosensing elements dependent on K^+_{ATP} channels in the mediobasal hypothalamus. Previous reports have indicated that the central regulation of glucagon release is not confined to the mediobasal hypothalamus but also involves the parabrachial nucleus (Fujiwara et al., 1988) and the suprachiasmatic nucleus (Fujii et al., 1989). Additionally, glucose-sensing neurons with K^+_{ATP} channels have been identified several extra-hypothalamic sites, including the nucleus of the solitary tract. (Dallaporta et al., 2000) where the channels are functionally related to the regulation of aspects of autonomic outflow (Ferreira et al., 2001). As such, it seems reasonable to conclude that hypoglycemic regulation of centrally mediated glucagon release may be controlled by multiple glucose-sensing sites, which collectively require functional K^+_{ATP} channels, as indicated by the Kir6.2$^{-/-}$ studies.

It is interesting that the current results show that, in spite of a robust glucagon response, hypothalamic administration of glibenclamide delayed the recovery from mild insulin-induced hypoglycemia. This impaired counter-regulatory response was associated with notable reduction in plasma norepinephrine levels. These data imply that noradrenergic sympathetic outflow was blocked by closure of hypothalamic K^+_{ATP} channels, and that this effect is sufficient to cause a reduction in the overall counter-regulatory glucose response, even in the presence of elevated glucagon and epinephrine levels. Such an interpretation is consistent with previous findings in which the magnitude of hepatic glucose output in response to stimulation by glucagon can be modified by factors of neural origin (Luers et al., 2000). The fact that central glibenclamide did not appear to alter epinephrine release may be consistent with the activation of mechanisms allowing for the differential control of sympathetic outflow (see Morrison, 2001). It should be noted that the epinephrine data are clearly affected by the experimental procedures in both treated and control animals, probably due to blood lost to sampling. Differential epinephrine responses due to the glibenclamide during hypoglycemia may have been masked.

In summary, the in vivo data presented in this paper offer support for the hypothesis that the physiological relevance of K^+_{ATP} channels in discrete regions of the brain are important in the mediation of glucose homeostasis. The evidence is supported by similar effects of two structurally distinct, but mechanistically similar pharmacologic agents, glibenclamide and repaglinide. The physiologic pathways but which alterations in the activities of hypothalamic K^+_{ATP} channel led to alterations in peripheral glucose homeostasis remain to be elucidated, but initial indications point to an involvement in the regulation of activity of the sympathetic nervous system, though addition mechanisms cannot be excluded.

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