The mechanisms underlying the unique pharmacodynamics of nateglinide

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Abstract

Nateglinide, a D-phenylalanine derivative, belongs to a new group of insulintropic agents with rapid onset and short duration of action. These agents have been developed to reduce the risk of hypoglycaemia associated with pharmacological control and to decrease the likelihood of pancreatic beta-cell exhaustion. Nateglinide mediates the release of insulin from beta-cells by binding to the sulphonylurea receptors, which leads to the closure of ATP-sensitive K⁺ channels. Increasing evidence from receptor binding, mechanistic and in vitro and in vivo insulin studies indicate unique pharmacodynamic and pharmacokinetic properties with nateglinide that are distinct from those of sulphonylureas. The time required by nateglinide to close beta-cell K_{ATP} channels is comparable to that of glyburide but threefold and fivefold faster than repaglinide and glimepiride, respectively. Furthermore, its effects are rapidly reversed with an off-rate at the K_{ATP} channel twice as fast as that of glyburide and glimepiride and five times faster than repaglinide. This results in a rapid and short insulin response characteristic of the physiological pattern of post-mealtime insulin release. Internalisation into beta-cells is not required for the action of nateglinide. Given that the kinetic profile of the agent is associated with selective enhancement of early-phase insulin secretion, nateglinide is expected to minimise post-meal hyperglycaemia with minimal propensity for hypoglycaemia. [Diabetologia (2003) 46[Suppl1]:M37–M43]

Keywords Type 2 diabetes, postprandial hyperglycaemia, insulin secretion, impaired glucose tolerance, sulphonylureas, insulin secretory responses.

Background

Postprandial glucose concentration is an important constituent of overall glucose exposure and postprandial hyperglycaemia poses an independent risk for developing macrovascular complications of diabetes [1]. Impaired kinetics of insulin secretion contribute to

postprandial hyperglycaemia in impaired glucose tolerance and in Type 2 (non-insulin-dependent) diabetes mellitus. In fact, progressive impairment of early insulin release accompanies the transition from normal to impaired glucose tolerance, and ultimately conversion to overt Type 2 diabetes [2]. An agent that improves or restores early insulin release would minimise postprandial hyperglycaemia and would thus be a valuable tool in the therapeutic armamentarium. However, the established insulintropic agents, i.e. sulphonylureas do not augment or replace early insulin release, rather they increase insulin concentrations throughout the day. Accordingly, sulphonylureas are relatively ineffective in controlling prandial glucose excursions. In contrast to sulphonylureas, nateglinide (an amino-acid derivative) and repaglinide (a meglitinide) are used as mealtime glucose regulators by vir-

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Abbreviations: SUs, Sulphonylureas; SUR, sulphonylurea receptor; Kir, inwardly-rectifying potassium channel; K_{ATP} ATP-sensitive potassium channel.

Fig. 1. Structures of insulintropic agents

tue of their mode of administration (pre-meal), their rapid absorption and elimination and their rapid and transient action as insulin secretion enhancers [3].

Nateglinide and repaglinide are ligands of the ‘sulphonylurea receptor’ (SUR), as are, of course, sulphonylureas (SUs) such as tolbutamide, glibenclamide and glimepiride. The SUR is a membrane receptor-effector system that comprises a ligand binding subunit (SUR1) and a pore-forming K^+ channel subunit, (Kir 6.2). The functional beta-cell K_{ATP} channel is composed of four subunits each of SUR1 and Kir 6.2, i.e. (SUR1/Kir 6.2)₄. Kir 6.2 forms the potassium pore in the membrane and SUR1 is needed for the proper function of the channel [4, 5]. Interaction of insulintropic ligands with the membrane SUR1 protein results in the closure of the K_{ATP} channel, membrane depolarisation and entry of extracellular calcium via the voltage-gated (L-Type) Ca^{++} channel. The increased intracellular free Ca^{++} concentration acts as a stimulus for exocytosis of insulin-containing secretory granules [6, 7].

Despite being effective insulin-secretion-enhancing ligands of SUR1, neither nateglinide nor repaglinide are sulphonylureas because (Fig. 1) neither agent contains the sulphonylurea moiety. The benzoic acid derivative, repaglinide, was derived through optimisation of the substituted benzoic acid, meglitinide, that is often described as the non-sulphonylurea moiety of glyburide. Thus, repaglinide is appropriately considered a meglitinide analogue [8]. Meglitinide is known to produce SU-like activity in vitro [9] and in vivo [10] by blocking K_{ATP} channels in pancreatic beta cells [11]. The hypoglycaemic activity of repaglinide resides predomi-

nantly in the S-enantiomer, in which the acidic (COOH) and amidic (CONH) groups are supposedly involved in binding to two putative sites of the SUR [12]. Specific hydrophobic differences between repaglinide and glyburide or glimepiride could be responsible for the differential binding affinities of these agents to the SUR site and, in turn, differential pharmacodynamics [13].

Nateglinide, N-[(trans-4-isopropylcyclohexyl)carbonyl]-D-phenylalanine, is the structurally optimal compound of a class of N-benzoyl-D-phenylalanines, discovered through a screen of amino acid derivatives in a search for rapid and short acting antidiabetic agents [14, 15]. The R- but not the S-enantiomer was found to be active in stimulating insulin release, and the carboxyl and the phenylmethyl moieties are essential for glucose-lowering activity. In addition, the change in the benzamide structure to the cyclohexylcarbamide and the introduction of an isopropyl group to the trans four-position of the cyclohexylcarbamide produced the highest potency. Because nateglinide was not derived from meglitinide it is not a meglitinide analogue, despite conformational analyses suggestive of similarities in three-dimensional structure [16].

The purpose of this article is to review pre-clinical studies that address the fast-on and fast-off natures of nateglinide and repaglinide with emphasis on basic mechanisms of action. Said mechanisms of nateglinide and repaglinide will be compared and contrasted, when possible, with those of SUs.

Interaction with the SUR

Nateglinide, repaglinide and the various SURs displace [³H]-glibenclamide from membranes of RIN-m5F cells and HEK293 cells expressing human

SUR1 [17]. The relative order of potency for displacement of [^3H]-glibenclamide in steady-state competitive binding experiments is consistent with a mode of action involving competitive interaction at SUR1. Neither nateglinide nor repaglinide have any effect on the dissociation kinetic parameters for [^3H]-glibenclamide. This result suggests that nateglinide and repaglinide compete for binding to the glibenclamide binding site on SUR1. The experimental data are also consistent with molecular modelling studies that propose an overlapping binding site for the sulphonylureas and repaglinide [13].

The inability to directly measure specific [^3H]-nateglinide binding using a variety of assay methods suggests that nateglinide dissociates rapidly from the sulphonylurea receptor [17]. This finding is consistent with the weak binding displayed by nateglinide in competitive binding experiments ($K_i=170$ nM for rat/RIN-m5F membranes and $K_i=1300$ nmol/l for the HEK293 human SUR1 expressing membranes). The estimated off-rate for nateglinide is about 34 min^{-1} ($t_{1/2}\sim 1$ s) compared to about 0.36 min^{-1} ($t_{1/2}\sim 2$ min) for repaglinide [17]. The marked differences in binding affinity and estimated half-life on the receptor might in part underlie kinetic differences in the pharmacology of nateglinide and repaglinide.

Nateglinide and the SUs showed Hill coefficients near unity (>0.85) in membrane binding studies in RIN-m5F cells. In contrast, the Hill coefficient for repaglinide is significantly lower, i.e. 0.60 ± 0.009 , which accords with the relatively low Hill coefficient (0.51 ± 0.04) with AZ-DF 265, an analogue of repaglinide, in competitive binding experiments also using intact RIN-m5F cells [18]. Two plausible interpretations of this are that the high affinity receptor population is heterogeneous [19], i.e. there are two forms of the high affinity SUR in the RIN-m5F membranes or there is a single SUR that can exist in two interchangeable states. Repaglinide is proposed to bind to the two forms or states of the receptor with different affinities whereas the other SUR1 ligands such as nateglinide do not. This explanation would also be consistent with the two-state model for the K_{ATP} channel proposed by J. Bryan et al. based on their observations of electrophysiological channel bursting behaviour. These authors have suggested that SUR1/2 regulates the transition of the K_{ATP} channel from a silent to a bursting state [5].

Interaction with K_{ATP} channel in beta cells

Following binding of insulinotropic ligands to the SUR1, the next stage in beta-cell stimulus-secretion coupling is the closure of ATP-sensitive K^+ channels (now known to be Kir 6.2), which reduces K^+ efflux and thus depolarises the membrane. The activity of K_{ATP} channels can be assessed directly using patch-

Table 1. Comparison of the kinetics of K^+_{ATP} -channel closure induced by nateglinide, glyburide, glimepiride and repaglinide in isolated rat beta cells (Reproduced with permission from [22])

Drug	$T_{1/2\text{-on}}^{\text{a}}$ (min)	$T_{1/2\text{-off}}^{\text{b}}$ (min)
Nateglinide	4.2 ± 0.2	29.7 ± 4.2
Glyburide	4.2 ± 0.5	$68.1\pm 4.0^{\text{c}}$
Glimepiride	$22.3\pm 1.8^{\text{c}}$	$62.2\pm 2.0^{\text{c}}$
Repaglinide	$12.2\pm 1.8^{\text{c}}$	$175.0\pm 1.0^{\text{c}}$

^a Time to elicit half-maximal closure

^b Elapsed time to 50% reversal

^c Significantly different from nateglinide. ($p<0.05$)

Data are means $\pm$ SEM, $n=5$ to 7 for each agent

clamp techniques [20]. Electrophysiological recording of the membrane potential of single rat pancreas beta cells with perforated whole-cell configuration showed that nateglinide ($10\text{ }\mu\text{mol/l}$) rapidly (<30 s) depolarised the membrane in the presence of sub-stimulatory glucose concentrations (2.8 mmol/l), and the effects were rapidly reversed when nateglinide was removed [21]. Recording of single channel current from cell-attached membrane patches confirmed a rapid and reversible effect of nateglinide to close K_{ATP} channel, with an IC_{50} of $0.23\text{ }\mu\text{mol/l}$ compared to $12.8\text{ }\mu\text{mol/l}$ with tolbutamide [21]. Additional patch-clamp studies of rat pancreatic beta cells, using either conventional or perforated whole cell configurations, with conditions optimised to eliminate any confounding contribution of channels other than the K_{ATP} channel to the whole-cell current, were also carried out to further explore the direct effects of nateglinide and other SUR ligands on K_{ATP} currents. Here, repaglinide was shown to close K_{ATP} channels with a potency very close to that of glimepiride, but marginally more potent than glyburide, whereas nateglinide was considerably less potent than the other drugs [17, 22].

Kinetic studies of these SUR ligands, at fixed equipotent concentrations ($2\times$ respective IC_{50} at 5 mmol/l glucose), unequivocally showed that the onset of the closure of K_{ATP} channels occurred rapidly with administration of nateglinide and the effect was promptly and completely reversed [17, 22]. The time to achieve half-maximal inhibition of K_{ATP} current ($T_{1/2\text{-on}}$) and the times to achieve 50% reversal ($T_{1/2\text{-off}}$) are summarised in Table 1. The time needed to close K_{ATP} channels by nateglinide is comparable to that of glyburide but is threefold and fivefold, respectively, faster than those of repaglinide and glimepiride, a property that reflects the relatively rapid onset of nateglinide's effect in vivo. Despite the similar inherent capability of glyburide and nateglinide to rapidly close K_{ATP} channels, glyburide is known to be slow-acting and long-acting in vivo, possibly because of slow absorption. Furthermore, the effects of nateglinide on K_{ATP} channels are more readily reversed when it is removed

than are those of sulphonylureas and repaglinide. The off-rate of nateglinide is twice as fast as that of glyburide and glimepiride and five times more rapid than that of repaglinide. Thus, the insulin secretory action of nateglinide is inherently shorter in duration than repaglinide, although the two compounds have similar pharmacokinetic profiles [3]. In addition, repeated application of nateglinide to beta cells with an interval of approximately 60 min blocked K_{ATP} channels to the same extent and with the same rapidity as the initial application [22]. This finding is consistent with maintenance of nateglinide's insulin secretory potential in vitro [23] and in vivo [24].

Internalisation contributes to differential kinetics

The difference in the kinetics underlying the respective insulin secretion enhancing effects of nateglinide and SUs could, in part, be attributable to differences in their relative capacity for insertion into the phospholipid domain of the plasma membrane versus transversion of the membrane. To exert their action, the SUs, glyburide and glimepiride, are known to require internalisation into beta cells [25, 26]; in contrast, nateglinide is not appreciably internalised [27]. The process of internalisation of SUs can be envisioned as introducing a time lag between exposure to the beta cell and stimulation of insulin release. Thus, the prolonged presence of SUs inside the cell, in contrast to the rapid clearance of nateglinide via the circulation, could well contribute to the longer duration of action of SUs at both the level of K_{ATP} channel closure and insulin secretion. It is still not clear, however, whether internalisation of repaglinide is required for stimulation of insulin secretion.

Insulin secretion in vitro

The effects of nateglinide have been studied in the isolated perfused pancreas of mice, rats, and hamsters, which allow examination of the kinetics of hormone secretion. Upon exposure to nateglinide (3 $\mu\text{mol/l}$), insulin release took place rapidly, peaked at 1 to 3 min and declined to baseline within 30 min [28]. Nateglinide (30 $\mu\text{mol/l}$) stimulated insulin release in isolated perfused rat pancreas in a biphasic manner and the response subsided immediately when the compound was removed. Although the response to glyburide (3 $\mu\text{mol/l}$) was similar in magnitude it was slower in onset, less clearly biphasic and failed to washout when the drug was removed [29]. Other studies with rat isolated perfused pancreas have showed the vastly different secretory patterns induced by nateglinide and repaglinide [30, 31]. In the presence of glucose the rapid and biphasic response to nateglinide and its immediate washout is clearly distinguishable from the

Fig. 2A, B. Effects of repaglinide (0.01 $\mu\text{mol/l}$, **A**) and nateglinide (A-4166, 1.0 $\mu\text{mol/l}$, **B**) upon insulin release from the rat pancreas perfused in the presence of 5.6 mmol/l glucose. The reported values represent the means $\pm$ SEM of four experiments in each case. The drugs were administered for 20 min, as indicated by the vertical dotted lines. A 16.7 mmol/l glucose stimulus was applied at the end of the experiments (vertical dotted line between min 85 and 86). Reproduced with permission from [30]

relatively slow monophasic response and incomplete washout of repaglinide (Fig. 2) [30].

Nateglinide was also shown to elicit a characteristic profile of biphasic insulin release from the in situ perfused hamster pancreas [32]. The effect of nateglinide (30 $\mu\text{mol/l}$) was similar in magnitude to that of 0.3 $\mu\text{mol/l}$ glyburide, but more rapidly manifested and reversed than that of glyburide. The effect of nateglinide appeared specific for the early-phase of insulin secretion, since the drug effectively reversed the diazoxide-induced inhibition of early-phase release while glyburide reversed suppression of the late phase only, further indicating different kinetics between the two drugs. The rapid on-off kinetics of nateglinide's insulin secretory response in vitro are thus entirely consistent with the unique characteristics of the molecular interaction with SUR 1/Kir 6.2 complex.

Insulin secretion in vivo

A number of studies have described the action of orally administered nateglinide on plasma insulin and glucose concentrations in rodents, especially in rats. In the fasted state the insulin-stimulating effect of nateglinide is remarkably rapid in onset and short in duration [33]. This rapid on-off pharmacodynamic profile allows nateglinide to reduce or eliminate glucose excursions (during glucose tolerance tests or meals) without sustained or delayed hyperinsulinaemia and/or hypoglycaemia.

When administered orally in normal fasted rats, maximal glucose-lowering by nateglinide and tolbutamide occurred 2 and 3 h after administration and euglycaemia was restored within 4 and 6 h, respectively [24]. In another study comparing the pharmacodynamics (effects on both glucose and insulin concentrations) of several insulintropic agents in chronically-cannulated normal rats, agents at similarly-effective doses (in terms of maximal glucose-lowering) showed markedly different pharmacodynamics following their oral administration [33] (Fig. 3). Nateglinide (60 mg/kg) stimulated insulin secretion, reached peak concentrations within 4 min and returned to baseline within 90 min. Correspondingly, the glucose nadir occurred within 45 min of nateglinide administration and euglycaemia was restored by 90 min post-dose. In contrast, after oral administration of glipizide (1 mg/kg) peak insulin concentrations were not observed until 60 min post-dose, and steady-state hyperinsulinaemia and hypoglycaemia were observed usually from 60 min onward. The pharmacodynamics of repaglinide (0.2 mg/kg) were intermediate between those of nateglinide and glipizide. Although maximum insulin concentrations occurred 10 min post-dose, hyperinsulinaemia and hypoglycaemia were sustained throughout the 120 min sampling period. Thus, glipizide and repaglinide are relatively long-acting in rodents.

Since nateglinide is a regulator of mealtime glucose excursions, much work was focussed on the effects of nateglinide during a glucose or meal challenge. In normal fasted rats given an i.p. glucose bolus (1 g/kg), nateglinide reduced glucose excursions (both peak concentrations and incremental area under the curve) in a dose-related manner with virtually no propensity to elicit frank hypoglycaemia [24]. The short-acting SU, tolbutamide, also reduced glucose excursions but elicited a pronounced and sustained hypoglycaemia even at doses that were less effective than nateglinide to reduce peak glucose concentrations. Glipizide and glyburide were considerably less effective than nateglinide in reducing glucose excursions but both produced substantial hypoglycaemia that did not resolve within the 6-h experimental period [24]. Similar results with nateglinide have been found in the monkey in vivo, as highlighted in this supplement [34], and in recent clinical studies [35, 36, 37].

Fig. 3A, B. Plasma glucose (A) and insulin (B) concentrations after administration of vehicle (open circles), nateglinide (60 mg/kg, closed squares), glipizide (1 mg/kg, closed triangles) and repaglinide (0.2 mg/kg, closed diamonds) to normal fasted rats. Compounds were administered by oral gavage at time 0 min. Means $\pm$ SEM, $n=7$ rats per group. Reproduced with permission from [33]

During meal tolerance tests done in normal rats with compounds administered before a meal, nateglinide eliminated mealtime glucose excursions while post-absorptive glucose concentrations paralleled those in the control rats. Insulin excursions were similar to those in the control rats except for a surge in pre-meal concentrations, suggesting that the elimination of mealtime glucose excursions is due, in part, to an increase in insulin concentrations before feeding. Glyburide failed to influence early insulin release and mealtime glucose excursions, but augmented post-mealtime insulin concentrations and caused delayed hypoglycaemia. Although, glipizide and repaglinide both increased early as well as total insulin exposure and prevented mealtime glucose excursions [33] they caused delayed hypoglycaemia. These findings not only attest to the importance of early insulin release to mealtime glucose control but also suggest that nateglinide will have an improved safety and tolerability profile and greater potential to exert better mealtime glucose control, than either short-acting or long-acting SUs or repaglinide.

Nateglinide was also found to be unique in its ability to elicit rapid but transient insulin secretion and mealtime glucose control in rodent models of insulin resistance and impaired glucose tolerance. During OGTTs done in rats fed with high-fat food and in Zucker fatty rats, nateglinide increased insulin concentrations more rapidly than did glipizide but elicited less overall hyperinsulinaemia. Accordingly, nateglinide was more effective than glipizide in reducing post-mealtime glucose excursions and had fewer propensities to elicit sustained, post-absorptive, hypoglycaemia [38].

Although limited information is available to compare insulin-secretion enhancers in other animal models, nateglinide was reported to elicit a more rapid and transient stimulation of insulin release than tolbutamide in normal dogs and to control post-mealtime glucose excursions more effectively than tolbutamide in the KK mouse, and glibenclamide in the n-STZ diabetic rat [39]. In summary, many animal studies have shown that the new kinetic profile of nateglinide is associated with selective enhancement of early-phase insulin secretion and as such, nateglinide is highly effective in minimising post-meal hyperglycaemia.

Conclusions

Nateglinide acts on the SUR1 and beta-cell K_{ATP} channels with unique kinetic properties, which translate to a fast and short-insulin secretory response. The drug, by restoring the normal physiology of post-mealtime insulin release characterised by a rapid onset and a brief duration, is expected to be especially effective in normalising post-mealtime hyperglycaemia and could actually correct the early beta-cell dysfunction in Type 2 diabetes, i.e. the loss of early-phase of insulin release. The action of nateglinide in animal models attests to the relative importance and effectiveness of early-phase insulin secretion, compared to late-phase insulin release, to post-mealtime glycaemic control. Thus, in restoring a more physiological pattern of early insulin secretion, nateglinide holds a unique therapeutic promise in blunting post-mealtime glycaemic excursions in Type 2 diabetes while preventing sustained or delayed hyperglycaemia and hyperinsulinaemia.

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