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Calcium signaling and secretory granule pool dynamics underlie biphasic insulin secretion and its amplification by glucose: experiments and modeling

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- MGP developed models, wrote computer code, performed simulations, analyzed data, and wrote the manuscript.
- AT wrote computer code and performed simulations.
- JCH performed experiments, analyzed data, and wrote the manuscript.

Running head:
Ca^{2+} signals and granule pools in biphasic insulin secretion

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42   Abstract (244 words)
43
44   Glucose-stimulated insulin secretion from pancreatic β-cells is controlled by a
45   triggering pathway culminating in calcium influx and regulated exocytosis of
46   secretory granules, and a less understood amplifying pathway that augments
47   calcium-induced exocytosis. In response to an abrupt increase in glucose
48   concentration, insulin secretion exhibits a first peak followed by a lower
49   sustained second phase. This biphasic secretion pattern is disturbed in diabetes.
50   It has been attributed to depletion and subsequent refilling of a readily-
51   releasable pool of granules or to the phasic cytosolic calcium dynamics induced
52   by glucose. Here we apply mathematical modeling to experimental data from
53   mouse islets to investigate how calcium and granule pool dynamics interact to
54   control dynamic insulin secretion. Experimental calcium traces are used as
55   inputs in three increasingly complex models of pool dynamics, which are fitted to
56   insulin secretory patterns obtained using a set of protocols of glucose and
57   tolbutamide stimulation. New calcium and secretion data for so-called staircase
58   protocols, where the glucose concentration is progressively increased, are
59   presented. These data can be reproduced without assuming any heterogeneity in
60   the model, in contrast to previous modeling, because of nontrivial calcium
61   dynamics. We find that amplification by glucose can be explained by increased
62   mobilization and priming of granules. Overall, our results indicate that calcium
63   dynamics contribute substantially to shaping insulin secretion kinetics, which
64   implies that better insight into the events creating phasic calcium changes in
65   human β-cells is needed to understand the cellular mechanisms that disturb
66   biphasic insulin secretion in diabetes.
67
68
69   Keywords
70   β-cells, pancreatic islets, calcium dynamics, exocytosis, mathematical model
71
72
73
74   Glossary
75   RRP: Readily releasable pool;
76   $[Ca^{2+}]_i$: cytosolic free calcium concentration

INTRODUCTION

Glucose-induced insulin secretion requires operation of two complementary mechanisms in pancreatic β-cells: an increase in the cytosolic free calcium concentration $[Ca^{2+}]_i$ that triggers exocytosis of insulin granules and actuation of an amplifying pathway that augments the exocytotic response to calcium (25). The amplifying signals derive from glucose metabolism, but their exact biochemical nature is still uncertain (15, 25, 33).

It has long been known that a rapid and sustained increase in blood glucose induces a biphasic rise in plasma insulin concentrations in normal human subjects (5, 7). This peculiar insulin kinetics is due to the biphasic dynamics of insulin secretion by pancreatic β-cells as calculated by C-peptide deconvolution (62, 63) and directly established by in vitro studies using isolated human islets (26, 56). Although produced only by unphysiologically rapid glucose stimulations, this biphasic insulin response of β-cells has attracted considerable attention because a low first phase has proved to be predictive of a deterioration of glucose homeostasis (8, 45, 54). In patients with impaired glucose tolerance or overt diabetes, both phases are impaired (19, 30, 52, 54) with sometimes (62, 64) though not always (19, 30) a greater impact on the first phase. Elucidation of the cellular mechanisms underlying biphasic insulin secretion thus has clinical implications. Achieving such a goal however rests on accessible experimental models.

A biphasic dynamics also characterizes glucose-induced plasma insulin changes (29, 46) and in vitro insulin secretion (11, 35, 37) in rodents. To explain the two phases of insulin secretion observed in the perfused rat pancreas, Grodsky (21, 22) proposed a model, where a limited pool of readily releasable insulin "packets" was secreted quickly to create the first phase, and subsequent refilling of the pool was responsible for the second phase. He also modeled the so-called staircase protocol where the glucose stimulus is increased in small steps, each giving rise to a first-phase-like peak of insulin, by assuming that the readily releasable insulin pool is heterogeneous, containing insulin packets with different glucose-thresholds (21). According to an alternative model, proposed by Cerasi et al. (6), the two phases of insulin secretion result from the interaction of inhibitory and potentiating signals with different kinetics. Subsequent studies (36, 47) compared the storage- and signal-limited models, found that both have caveats, and concluded that a combined model with both limited insulin pools and time-dependent signals performed better. However, the cellular origin of the heterogeneity of the releasable pool of insulin and the biochemical nature of the putative inhibitory and potentiating signals remained elusive.

Studies of exocytosis in single β-cells provided substantial support to the pool model, with depletion of a readily releasable pool (RRP) yielding the first phase, while refilling of the RRP creating the second phase (2, 10, 48, 58). It was further suggested that cell-to-cell heterogeneity seen in $[Ca^{2+}]_i$ imaging experiments (32) could underlie the postulated threshold distribution for the RRP (50). Although β-cell coupling through gap-junctions within islets reduces intercellular heterogeneity substantially (59, 60) and synchronizes cellular

126 responses (49, 55), recent evidence indicates that some heterogeneity persists
127 between β-cells and islets (3, 39), possibly accounting for RRP heterogeneity.
128
129   The most obvious signal capable of inducing biphasic insulin secretion is
130 the triggering $[Ca^{2+}]_i$, the increase of which follows a biphasic kinetic in glucose
131 stimulated β-cells (13, 25, 28). Such a view is supported by experiments showing
132 that all maneuvers interfering with the rapid rise in $[Ca^{2+}]_i$ alter the first phase
133 and that all agents inducing a rapid $[Ca^{2+}]_i$ rise induce a rapid secretion (25, 28).
134 However, against this interpretation speak observations of biphasic insulin
135 secretion in face of virtually sustained elevations of $[Ca^{2+}]_i$ produced by
136 tolbutamide or KCl (28, 43). Whether amplifying signals and or depletion of a
137 limited amount of releasable insulin contribute to the phasic insulin pattern
138 under these conditions is unclear.
139
140   In the present study we combined experimental measurements of $[Ca^{2+}]_i$
141 and insulin secretion in mouse islets with mathematical modeling to unravel the
142 contributions of $[Ca^{2+}]_i$ signals and pool dynamics to biphasic insulin secretion.
143 Our analysis shows that triggering signals and granular pools both contribute to
144 shape the biphasic release pattern, and uncovers mechanisms underlying
145 amplification by glucose of the secretory response to calcium.
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148
149 **MATERIALS AND METHODS**
150
151 *Experiments*
152
153 All experiments were performed with islets isolated from the pancreas of female
154 C57BL6 mice. After hand selection, the islets were cultured overnight in RPMI
155 medium containing 10 mM glucose, and then used for dynamic measurements of
156 insulin secretion or $[Ca^{2+}]_i$. All methods were exactly as described in our
157 previous studies (29, 43). Because all presented traces correspond to averages
158 of results obtained with several islets, oscillations in $[Ca^{2+}]_i$ and insulin secretion
159 present in individual islets are masked, whereas the biphasic dynamics of these
160 responses are preserved.
161
162 *Mathematical modeling*
163
164 Our aim was to study the impact of $[Ca^{2+}]_i$ dynamics on insulin secretion. We
165 developed various models of granule pool dynamics that were driven by the
166 experimentally recorded $[Ca^{2+}]_i$ traces (see Fig. 1). Simulated secretion profiles
167 were then fitted to experimental insulin patterns to investigate which models
168 were able to fit the data satisfactorily. The models were described by ordinary
169 differential equations, where some of the transition rates between different
170 pools depended on the glucose concentration, whereas the combined
171 exocytosis/secretion rate depended on the time-varying experimentally
172 recorded $[Ca^{2+}]_i$ traces. Parameter estimation was not our scope, and
173 identifiability issues and estimation accuracy were neglected.

174
175 <u>Model 1</u>
176 In this model, only a readily-releasable pool (*RRP*) is present. This pool is refilled
177 by a "mobilization" or "refilling" process with rate $M(G)$ depending on the
178 glucose concentration G from an infinite reserve pool. The RRP granules can
179 undergo fusion and secretion with rate $S(Ca)$ depending linearly on $[Ca^{2+}]_i$ above
180 a threshold (31, 49). Granules can also undergo glucose-independent
181 "internalization" or "loss-of-release-capability" with rate N from the RRP. The
182 instantaneous secretion is thus $RRP(t)* S(Ca(t))$. We assume that insulin I to be
183 fitted to the experimental data is measured from a reservoir described by first-
184 order kinetics with a time-constant of 1 min, i.e.,
185 $$dI/dt = (S * RRP - I) / (1 \text{ min}).$$
186 This model has 10 parameters to be estimated.
187
188 <u>Model 2</u>
189 This model adds an intermediate pool X located near or at the plasma membrane
190 to model 1. The granule pool X is refilled from an infinite reserve pool with rate
191 $M(G)$ depending on the glucose concentration G. From the pool X, the granules
192 enter the RRP following glucose-dependent priming with rate $p(G)$. As in model
193 1, the RRP granules may be released with rate $S(Ca)$. Granules can also undergo
194 glucose-independent "unpriming" with rate q from the RRP, and glucose-
195 independent internalization with rate N from X. Model 2 has 18 parameters to be
196 estimated.
197
198 <u>Model 3</u>
199 In this model, glucose-independent mobilization directly to, and internalization
200 from, the RRP with rate k, respectively l, was added to Model 2. Such direct
201 mobilization bypassing the pool X may represent "basal" mobilization and is
202 accessible to tolbutamide- or potassium-induced $[Ca^{2+}]_i$ elevations. Model 3 has
203 20 parameters to be estimated.
204
205
206 ***Data fitting***
207
208 For each model, we fixed the parameters and simulated 14 different protocols
209 corresponding to the experimental data. The parameters M and p were allowed
210 to change with glucose levels (assuming the same value for 10 mM and 11.1 mM
211 glucose to reduce the number of parameters to fit). For experiments with pre-
212 stimulation in 3 mM glucose (rows 1, 3 and 4 in Fig. 2), the initial conditions for
213 the pool sizes were set so that the model was in steady-state in the absence of
214 secretion. For experiments with pre-stimulation in 8.5 mM glucose (row 2 in Fig.
215 2), the initial conditions were set to the final value of the model simulation after
216 a step from 3 mM to 8.5 mM glucose.
217
218 The parameters were then varied automatically within the optimization
219 algorithm, and the simulated secretion data were compared to the experimental
220 recordings to minimize the squared error, calculated as the difference between
221 simulated (*I*) and experimental insulin data for the 14 protocols. In order to
222 exploit the information from the relatively few tolbutamide protocols, we

223   weighted the residuals from fitting of the experiments from Mourad et al. (43) 10
224   times higher.
225
226   The procedure was repeated with different initial choices of the
227   parameter set, to reduce the risk of ending in a local minimum, and eventually
228   led to a single parameter vector for which the model fit to the 14 experimental
229   data sets was optimal. Model parameters were constrained so that mobilization
230   and priming were non-decreasing functions of glucose. In other fits no
231   constraints were imposed, allowing mobilization and priming rates to be non-
232   monotone functions of glucose, hence permitting extra degrees of freedom for
233   the estimation of parameters.
234
235   Simulations were done in MATLAB (version R2017b; Mathworks Inc.)
236   using the ode45 solver. Fitting was performed with the fmincon function. The
237   computer code is available at http://www.dei.unipd.it/~pedersen
238
239

240   **RESULTS**

241

242   ***Experimental data description***

243

244   We fitted our mathematical models to reproduce results from previously
245   published (29, 43) and novel studies of phasic islet $[Ca^{2+}]_i$ changes and insulin
246   secretion in response to different protocols of glucose or tolbutamide
247   stimulation (Fig. 2).

248

249   In a first series of experiments, taken from (29), brisk jumps of glucose
250   from 3 mM to 8.5, 11.1, 16.7 or 30 mM in perifusion medium resulted in biphasic
251   insulin secretion and $[Ca^{2+}]_i$ elevation (Fig. 2, A-D). The first phases of secretion
252   and $[Ca^{2+}]_i$ increased in both amplitude and duration with increasing glucose
253   concentration. Second phases of secretion and $[Ca^{2+}]_i$ also increased with
254   glucose. In a second series, islets were initially exposed to 8.5 mM glucose before
255   being stimulated with 11.1, 16.7 or 30 mM glucose (Fig. 2, E-G). Both insulin and
256    $[Ca^{2+}]_i$ responses were again biphasic, but first phases were smaller than after
257   initial perifusion in 3 mM glucose, whereas second phases were similar.

258

259   A third series of experiments, taken from (43), compared insulin and
260    $[Ca^{2+}]_i$ responses in islets subjected to stimulation with 15 mM glucose or 500
261   µM tolbutamide in 3 mM glucose (Fig. 2, H-K). Salient differences and similarities
262   were identified. Sustained stimulation with either stimulus induced a clearly
263   biphasic secretion of insulin although the dynamics of the $[Ca^{2+}]_i$ response
264   evoked by tolbutamide was hardly biphasic compared to that evoked by glucose
265   (Fig. 2, H vs. I). Tolbutamide-induced secretion was about 50% smaller than
266   glucose-induced secretion in the face of a slightly greater elevation of $[Ca^{2+}]_i$, a
267   difference that reflects amplification of insulin secretion by glucose. Application
268   of short pulses of tolbutamide or glucose, to mimic several first phases, again
269   induced roughly similar $[Ca^{2+}]_i$ responses but smaller insulin responses with
270   tolbutamide than glucose (Fig. 2, J vs. K). With each stimulus, the amplitude of
271   the first insulin pulse was slightly larger than that of subsequent pulses.

272
273
274   Finally, in a series of novel experiments, islets were stimulated using
275 staircase increases in glucose concentration from 3 to 7, 10 and eventually 15
276 mM (Fig. 2, L-N). When steps at 7 and 10 mM glucose were short (5 min), a
277 distinct $[Ca^{2+}]_i$ peak was produced by every increase in glucose concentration,
278 which was accompanied by a peak of insulin secretion (Fig. 2L). Applying longer
279 glucose steps (20 min) did not substantially change the pattern (Fig. 2M); a small
280 second phase evolved at 7 and 10 mM glucose, but the peaks evoked by each
281 increase in glucose had a similar size to the ones evoked by short steps. Notably,
282 the first phase of the $[Ca^{2+}]_i$ response to 15 mM glucose was much longer, though
283 not greater in amplitude, following the single step directly from 3 mM glucose
284 than during the staircase protocol (step from 10 mM glucose) and the
285 corresponding first phase of insulin secretion was considerably larger (Fig. 2N).
286 Second phases were similar. Omission of extracellular calcium, while keeping
287 glucose at 15 mM, markedly lowered islet $[Ca^{2+}]_i$ and stopped insulin secretion.
288 Reintroduction of calcium elicited rapid increases in $[Ca^{2+}]_i$ and secretion, but
289 the insulin peak was smaller than that observed after a step from 3 to 15 mM
290 glucose although the $[Ca^{2+}]_i$ response was not smaller (Fig. 2N).
291
292   We next used these 14 experimentally recorded $[Ca^{2+}]_i$ traces as inputs to
293 models of insulin release. The simulated secretion profiles were then fitted to
294 the corresponding experimental insulin patterns. As explained above, three
295 models of increasing complexity were compared.
296
297

298 *Performance of Model 1*

299
300 The simple Model 1, with a single pool, fitted the data acceptably but
301 underestimated the peaks when glucose was stepped from 3 mM to 8.5, 11.1,
302 16.7 or 30 mM (Fig. 3, A-D, red curves), and overestimated the peak after
303 reintroduction of calcium in 15 mM glucose (Fig. 3, L and N). This latter
304 discrepancy could be corrected (not shown), in this and in Models 2 and 3 to be
305 discussed below, by assuming lower refilling rate at low $[Ca^{2+}]_i$ levels (23).
306 During stimulation with 8.5 mM glucose, the RRP was nearly constant (Fig. 4A).
307 The smaller size of the peaks observed when stepping to 16.7 or 30 mM glucose
308 occurred from 8.5 mM (Fig. 3F and G) rather than 3 mM glucose (Fig. 3C and D)
309 was almost entirely due to the shorter duration of the first-phase $[Ca^{2+}]_i$ signals
310 following pre-exposure to 8.5 mM glucose (Fig. 2).
311
312   As shown in Fig. 2 (panels H-K), tolbutamide in 3 mM glucose evoked
313 slightly larger increases in $[Ca^{2+}]_i$ than did 15 mM glucose, but the resulting
314 secretion of insulin was larger with glucose than tolbutamide; that difference
315 was observed during the two phases of a sustained stimulation and during
316 application of repetitive pulses. Model 1 reproduced these differences
317 reasonably, though the first peak of tolbutamide-stimulated secretion was
318 slightly overestimated (Fig. 3H and J), and the first phase of secretion triggered
319 by 15 mM glucose slightly underestimated (Fig. 3I and K) in the model.
320

321   Model 1 was also able to reproduce the staircase experiments (Fig. 3L and
322   M). In response to each step in glucose, simulated insulin secretion showed a
323   peak, which was driven by $[Ca^{2+}]_i$ dynamics, not pool depletion, since the RRP
324   was nearly constant during the staircase protocols (Fig. 4F). This contrasts with
325   Grodsky's model (21), which postulated heterogeneity of the RRP, the peak of
326   secretion induced by each glucose step being attributed to release of subpools of
327   granules with increasing glucose-thresholds. In our model these insulin peaks
328   were purely due to the peaks in the $[Ca^{2+}]_i$ signal (Fig. 2L and M).
329
330   The estimated refilling rate remained at the basal value up to ~10 mM
331   glucose, after which it increased, yielding an overall sigmoidal dependence on
332   glucose concentration (Fig. 3O, red curve). When the constraint that refilling as a
333   function of glucose be non-decreasing throughout was removed, no noticeable
334   improvement in the model fits was observed, and the overall results were as
335   described above (Fig. 3, blue curves).
336
337   Thus, within the physiological range of glucose concentrations, Model 1
338   predicted that differences in secretion measured in the various protocols were
339   largely due to the different $[Ca^{2+}]_i$ signals. Pool depletion played a role at higher
340   glucose levels and during tolbutamide stimulation (Fig. 4B, D and E).
341
342   In the model, tolbutamide acted (via Ca^{2+}) only on secretion $S(Ca)$ and the
343   relatively low peak of secretion in response to tolbutamide (Fig. 2H and J)
344   imposed a limit on the estimate of the initial size of RRP. A limited pool in turn
345   resulted in a low simulated peak when glucose was stepped to 8.5 mM (Fig. 3A),
346   since the refilling rate M could not be too large in order for the model to
347   reproduce the nearly absent second phase of secretion at 8.5 mM. The same
348   problem was seen at the other levels of glucose. In other words, in this simple
349   Model 1 there was a contradiction between the low peak of secretion seen in
350   response to tolbutamide and the relatively large first phase of secretion in
351   response to glucose. We therefore analyzed the results with a slightly more
352   complicated model.
353
354
355   ***Performance of Model 2***
356
357   Compared to Model 1, Model 2 has an additional intermediate pool X between
358   mobilization and the RRP (see Fig. 1), which could correspond to docked but
359   unprimed granules (9, 13, 16, 65). This model simulated most data sets well,
360   except for the insulin peaks following glucose steps from 3 mM to 8.5 or 11.1
361   mM, which were much larger in the experiments compared to the simulated data
362   (Fig. 5A and B, red curves). This discrepancy was caused by the restrictions on
363   the priming and mobilization rates, which were imposed to be non-decreasing
364   functions of glucose. Indeed, when this constraint was removed, Model 2 was
365   able to fit the data much better, which resulted in a U-shaped glucose-
366   dependence of the mobilization rate (Fig. 5, blue curves).
367
368   When priming and mobilization rates were constrained to be non-
369   decreasing functions of the glucose concentration, the priming and refilling rates

370   were estimated to be low (Fig. 5O). This assured that 8.5 mM glucose did not
371   increase the RRP (Fig. 6A) to avoid that a subsequent rise in the $[Ca^{2+}]_i$ signal led
372   to a too large insulin peak when glucose was raised further (Fig. 5, E-G). Since the
373   priming rate at 8.5 mM is small (Fig. 5O), precluding recruitment from the
374   intermediate pool X (Fig. 6A, thin line), the simulated first phase at 8.5 mM
375   glucose was small compared to the experiments (Fig. 5A).
376
377   The simulated first peak of insulin was larger upon stepping from 3 to
378   11.1 rather than 8.5 mM glucose (Fig. 5, B vs. A) because the priming rate was
379   increased. However, to fit the still low rate of second-phase secretion measured
380   in 11.1 mM glucose, the increase in mobilization and priming rates had to be
381   limited in the model (Fig. 5O). This explains why the simulated first peak
382   remained lower than the experimental first phase (Fig. 5B). At higher glucose
383   concentrations the fits were excellent, except for a minor discrepancy when
384   stepping from 8.5 mM to 30 mM glucose (Fig. 5G), where the experimental
385   trough following the first phase was absent in the model fit. This discrepancy is
386   related to the rising second phase of secretion in the data, which the model is
387   unable to capture. Similarly to Model 1, the RRP and the pool X were nearly
388   constant during 8.5 mM glucose stimulation (Fig. 6A). The smaller secretory
389   responses to 16.7 or 30 mM glucose observed after pre-exposure to 8.5 rather
390   than 3 mM glucose (Fig. 5F and G vs C and D) were mainly due to differences in
391   the Ca^{2+} responses.
392
393   Fits to experimental data obtained during constant or intermittent
394   stimulations with tolbutamide or 15 mM glucose were excellent (Fig. 5, H-K). In
395   particular, the second, third and fourth pulses were reduced compared to the
396   first ones (Fig. 5J and K) because the intermediate pool X and the RRP only
397   refilled partly between stimuli (Fig. 6, D and E). Also, the staircase experiments
398   were reproduced very well (Fig. 5L and M).
399
400   According to Model 2, estimated priming and mobilization rates were
401   glucose-independent up to ~10 mM (Fig. 5O), suggesting that changes in the
402    $[Ca^{2+}]_i$ profiles were entirely responsible for the differences in secretion within
403   this physiologically relevant range.
404
405
406   ***Performance of Model 3***
407
408   Model 3 has an additional glucose-independent path refilling the RRP. The model
409   fitted the data very well, even when priming and mobilization rates were
410   constrained to increase with glucose (Fig. 7, red curves). No significant
411   improvement was obtained when this assumption was relaxed (Fig. 7, blue
412   curves). This model was able to reproduce the substantial peaks of secretion
413   when stepping from 3 mM to 8.5 or 11.1 mM glucose and above (Fig. 7, A-D).
414   Stimulation with 8.5 mM glucose depleted the intermediate pool X almost
415   completely (Fig. 8A), because priming was stimulated but refilling was not at this
416   glucose level. Subsequent steps in glucose therefore led to smaller first-phase
417   peaks, since first-phase secretion was mostly due to glucose-dependent
418   recruitment of X. Thus, in contrast to the two previous models, Model 3

419   attributed to pool depletion (of *X*) a major role in explaining the differences
420   following 3 mM or 8.5 mM pre-exposure.
421
422   As for Model 2, tolbutamide and staircase experiments were reproduced
423   well, although Model 3 slightly overestimated the first peaks in response to the
424   steps to 7 mM and 10 mM glucose (Fig. 7L and M).
425
426   Compared to Model 2, the estimated priming rate increased with glucose
427   already below 10 mM (Fig. 7O), suggesting that both granule and $[Ca^{2+}]_i$
428   dynamics played a role in shaping release patterns under physiological
429   conditions. Indeed, depletion of *X* because of rapid priming (Fig. 8A) allowed the
430   model to reproduce the peak of secretion already at 8.5 mM (Fig. 7A). These
431   estimates for the glucose-dependence of the priming rate in Model 3 correspond
432   to experimental findings that amplification operates already at low glucose
433   concentrations that do not increase $[Ca^{2+}]_i$ and, therefore, do not trigger insulin
434   release on their own (25).
435
436   In contrast to Model 2, the pool size of *X* was nearly constant during
437   tolbutamide simulations (Fig. 8D) since the additional direct refilling route
438   allowed the model to refill the RRP between tolbutamide pulses with a very low
439   priming rate *p* at 3 mM glucose. This in turn permitted a very low depriming rate
440   *q*, which was estimated to be ~10 fold lower in Model 3 compared to Model 2.
441   With lower *q* and basal *p*, Model 3 was able to create large simulated peaks when
442   glucose was raised, by rapid glucose-dependent recruitment of the intermediate
443   pool *X* (Fig. 8A, B and E). In other words, tolbutamide acted only on the RRP,
444   whereas glucose acted on both *X* and RRP by recruiting *X* rapidly into the RRP,
445   thereby causing the two pools to behave as if they were one. Amplification by
446   higher concentrations of glucose was caused by increased mobilization of
447   granules into *X* followed by very rapid priming into the RRP.
448
449
450   **DISCUSSION**
451
452   Since it is notoriously more difficult to obtain long $[Ca^{2+}]_i$ recordings from human
453   than mouse islets, and hence to study how calcium influences insulin secretion
454   patterns through interactions with granule dynamics, we applied our modeling
455   approach to previously published and novel data from mouse islets. Our analysis
456   shows that both $[Ca^{2+}]_i$ changes and insulin granule pools contribute to biphasic
457   secretion. Our findings give biological identity to the phenomenological signals
458   proposed by Cerasi et al. (6) in the form of intracellular Ca^{2+}, while the pool
459   description is virtually as suggested from experiments (2, 48).
460
461   The mathematical models presented here give a coherent framework for
462   the integration of $[Ca^{2+}]_i$ and granule pools, and are in some sense an updated
463   version of an earlier signal-pools-model (36). Notably, we did not assume any
464   heterogeneity of the RRP, in contrast to some earlier pool models reproducing
465   the staircase protocol (21, 50). Although our pool models are rather simple
466   compared to previous, detailed but less data-driven, models of granule pool
467   dynamics (4, 9, 51), they have the advantage on being driven by measured $[Ca^{2+}]_i$

468   profiles. Pedersen and Sherman (51) also included phasic and oscillatory "$[Ca^{2+}]_i$
469   profiles" consisting of square pulses, but with no attention to the glucose
470   dependency. Grespan et al. (20) similarly modeled calcium phenomenologically
471   in combination with the description of a single granule pool.

472

473   Using Model 2 and Model 3, which fitted the data best, we can investigate
474   the mechanisms creating the first phase of insulin release. Upon stimulation with
475   moderately elevated glucose concentrations (<10 mM), the initial $[Ca^{2+}]_i$ peak is
476   short and, in both models, a decline of the triggering signal terminates the first
477   phase of secretion, although the RRP is still not completely depleted (Figs. 6A
478   and 8A). A relevant difference between the two models is that, at moderate
479   glucose levels, the intermediate pool X is left nearly untouched in Model 2 (Fig.
480   6A) whereas it is rapidly recruited into the RRP in Model 3 (Fig. 8A), which
481   consequently allows this model to create a larger insulin peak than Model 2 in
482   response to moderate glucose concentrations.

483

484   At higher glucose concentrations, a longer first phase of $[Ca^{2+}]_i$
485   contributes to increase the first phase of secretion. However, secretion rates
486   start to decline sooner than $[Ca^{2+}]_i$ (Fig. 2) (29), which might reflect progressive
487   depletion of the RRP. In both models, the intermediate pool X is rapidly depleted
488   by steps to glucose concentrations above 10 mM (Figs. 6B and 8B), which
489   temporarily increases the size of the RRP, and consequently augments the peaks
490   of secretion. The secretion peak is then terminated by partial depletion of the
491   RRP (Figs. 6B and 8B). In addition, granule "mobilization" towards pool X
492   augments with glucose above 10 mM (Figs. 5O and 7O), which mainly permits
493   setting of the second phase. Since $[Ca^{2+}]_i$ also increases with glucose both
494   mechanisms contribute to augment the second phase of secretion at higher
495   glucose. Thus, both the $[Ca^{2+}]_i$ signal and refilling of the pool of releasable
496   granules, which the signal acts on, are enhanced by glucose. In contrast,
497   tolbutamide does not increase the size of the RRP but acts only via calcium.

498

499   The mechanisms generating phasic $[Ca^{2+}]_i$ changes in response to a
500   glucose step are incompletely understood. Electrical activity is also biphasic
501   under these conditions, (24, 40, 42) and underlies the biphasic rise in $[Ca^{2+}]_i$.
502   Based on the prominent role of $[Ca^{2+}]_i$ in driving the release patterns under the
503   protocols investigated here, we encourage further studies on the mechanisms
504   involved in shaping first-phase electrical and $[Ca^{2+}]_i$ responses in healthy and
505   diabetic human β-cells.

506

507   Notably, we are able to simulate the stair-case protocol without assuming
508   any heterogeneity of the RRP. Each step of glucose is known to elicit a rapid
509   increase in electrical activity (41) that causes a new $[Ca^{2+}]_i$ peak above the
510   already elevated $[Ca^{2+}]_i$ (32). In response to the step to 7mM glucose, the $[Ca^{2+}]_i$
511   peak is too short for complete depletion of granule pools (Figs. 4F, 6F, 8F), and
512   the trough following the first phase of secretion is due to fading of the triggering
513    $[Ca^{2+}]_i$ signal, not to pool depletion. Hence, the RRP is still nearly filled when the
514   next glucose step creates a new $[Ca^{2+}]_i$ peak, which – therefore – can produce
515   another peak of insulin secretion. This interpretation does not exclude that cell-
516   to-cell heterogeneity (3) – in particular with respect to $[Ca^{2+}]_i$ amplitude,

517 amplifying signals, or the time to cell activation (32, 61) – contributes to this
518 pattern (21, 50), but highlights that the secretion profile of the staircase protocol
519 can be largely explained by $[Ca^{2+}]_i$ dynamics. Further modeling studies should
520 aim to investigate how cell-to-cell heterogeneity in combination with dynamic
521 $[Ca^{2+}]_i$ patterns shape insulin secretion from a population of β-cells.
522
523   Glucose-dependent amplification of calcium-induced exocytosis is
524 thought to account for 50% of insulin secretion during the two phases of insulin
525 secretion in mouse (25, 43) and human islets (27). We suggest that amplification
526 of first-phase insulin secretion is caused by glucose-dependent priming of
527 granules located at or very close to the plasma membrane, likely by recruitment
528 of exocytotic proteins to the insulin granules (1, 16, 18, 65). Second-phase
529 amplification is attributed mostly to glucose-dependent "mobilization" in
530 addition to rapid priming (17). It should however be kept in mind that
531 amplification does not require a functional cytoskeleton and that "mobilization"
532 does not imply long-distance transfer of granules (43, 44). The nature of the
533 amplifying signals generated by glucose metabolism is still a matter of debate,
534 but there is evidence for rapid ATP-dependent priming in single-cell recordings
535 (14), and several other products have been suggested to be involved the second-
536 phase amplification (15, 33).
537
538   Detailed studies of the dynamics of insulin secretion by islets from Type 2
539 diabetic subjects have yet to be performed. However, perifusions of normal
540 human islets have shown that increases in the pre-stimulatory glucose
541 concentration from 3 to 6, 8 or 10 mM progressively decrease the magnitude of
542 the first phase of insulin secretion induced by 15 mM glucose (26) but augment
543 the response to tolbutamide (27). A decrease in first phase was also observed in
544 mouse islets when stepping from 8 rather than 3 mM glucose to 16 mM (Fig 2C
545 vs. F). Our models indicate that both a smaller Ca^{2+} signal and – for Model 3 –
546 reduced refilling of the RRP account for the phenomenon. We, therefore, only
547 partly agree with a recent suggestion that defects in pool refilling explain the loss
548 of first phase and the decrease of second phase insulin secretion in Type 2
549 diabetic subjects, with no need to assume disturbed $[Ca^{2+}]_i$ handling (20). There
550 is evidence that insulin granule docking and priming are disturbed in diabetic β-
551 cells (17, 18). However, arginine (38, 53) and tolbutamide (34) remain able to
552 induce a peak of insulin secretion in diabetic patients, and in islets from diabetic
553 donors in vitro (12), an effect mimicked by KCl-induced depolarization in single
554 diabetic β-cells (10, 17). These results indicate that the RRP is not empty in
555 diabetic β-cells. We, therefore, believe it unlikely that the loss of biphasic insulin
556 secretion in diabetic subjects is the result of disturbed granule dynamics only. In
557 contrast to tolbutamide, glucose is poorly able to induce electrical activity in
558 diabetic compared to healthy β-cells (57), which most likely causes disturbed
559 $[Ca^{2+}]_i$ dynamics and blunted insulin secretion (28, 57).
560
561   In summary, we propose that phasic $[Ca^{2+}]_i$ patterns contribute
562 substantially to the creation of biphasic insulin secretion patterns, in addition to
563 granule dynamics. Hence, to understand the cellular mechanisms that lead to
564 disturbed biphasic insulin release in type 2 diabetes, better insight into the

565 generation of phasic electrical activity and $[Ca^{2+}]_i$ dynamics in human β-cells is
566 needed.
567
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571
572
573 <u>Disclosures</u>
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575
576
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580
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583
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830
831
832
833

834   **FIGURE LEGENDS**

835

836

837   Fig. 1: **Overview of the three considered models.**

838

839

840   Fig. 2:

841   **Experimental data.** Experimental $[Ca^{2+}]_i$ traces (in nM; thick curves, left axes)

842   and insulin secretion measurements (% of islet content per minute; thin curves

843   with dots, right axes). Time is in minutes. The data in panels A-G are from

844   Henquin et al. (ref 29), where glucose (G) was stepped at 0 min (indicated by

845   arrow) from 3 mM (A-D) or 8.5 mM (E-G) to 8.5, 11.1, 16.7 or 30 mM, as

846   indicated. The data in panels H-K are from Mourad et al. (ref 43), where islets

847   were stimulated by either 500μM tolbutamide (Tolb) in 3mM glucose or 15 mM

848   glucose, continuously or in 8 min pulses, as indicated by arrows. The data in

849   panels L-N are new experiments shown as means for 3 experiments of insulin

850   secretion and 12-18 islets for $[Ca^{2+}]_i$. In panel L, staircase increase in glucose

851   from 3 mM to 7 mM, 10 mM and finally 15 mM, in 5 min steps indicated by

852   arrows, followed by removal of extracellular calcium between 30 and 40 min

853   (indicated by the bar). In panel M, the steps of the staircase were extended to a

854   duration of 20 min, as indicated by arrows. In panel N, glucose was stepped from

855   3 mM to 15 mM at 0 min (arrow), followed by removal of extracellular calcium

856   between 15 and 25 min (bar).

857

858   Fig. 3:

859   **Model 1 results**. Simulated secretion profiles (% of islet content per minute;

860   axes are shown only once for each row) obtained with Model 1 fitted to the

861   experimental data (black dots and curves), either with (red curves) or without

862   (blue curves) the constraint of refilling being a non-decreasing function of the

863   glucose concentration G. Layout as in Fig. 2. Panel O shows parameters for

864   refilling ($M(G)$ in % of islet content per minute) with (red) or without (blue) the

865   monotonicity constraint on $M(G)$.

866

867   Fig. 4:

868   **Pool dynamics in Model 1.** Simulated dynamics of RRP (in % of islet content)

869   obtained with Model 1 for selected protocols as indicated.

870

871   Fig. 5:

872   **Model 2 results**. Simulated secretion profiles (% of islet content per minute;

873   axes are shown only once for each row) obtained with Model 2 fitted to the

874   experimental data (black dots and curves), either with (red curves) or without

875   (blue curves) the constraint of refilling and priming being a non-decreasing

876   function of the glucose concentration G. Layout as in Fig. 2. Panel O shows

877   parameters for priming ($p(G)$ with unit 1/min; upper, right axis) and

878   mobilization ($M(G)$ in % of islet content per minute; lower, left axis) with (red)

879   or without (blue) the monotony constraint on $p(G)$ and M(G).

880

881

882

883   Fig. 6:
884   **Pool dynamics in Model 2.** Simulated dynamics of RRP (thick curves; % of islet
885   content) and X (thin curves; % of islet content) obtained with Model 2 for
886   selected protocols as indicated.
887
888
889   Fig. 7:
890   **Model 3 results**. Simulated secretion profiles (% of islet content per minute;
891   axes are shown only once for each row) obtained with Model 3 fitted to the
892   experimental data (black dots and curves), either with (red curves) or without
893   (blue curves) the constraint of refilling and priming being a non-decreasing
894   function of the glucose concentration G. Layout as in Fig. 2. Panel O shows
895   parameters for priming ($p(G)$ with unit 1/min; upper, right axis) and
896   mobilization ($M(G)$ in % of islet content per minute; lower, left axis) with (red)
897   or without (blue) the monotony constraint on $p(G)$ and M(G).
898
899   Fig. 8:
900   **Pool dynamics in Model 3.** Simulated dynamics of RRP (thick curves; % of islet
901   content) and X (thin curves; % of islet content) obtained with Model 3 for
902   selected protocols as indicated.
903
904
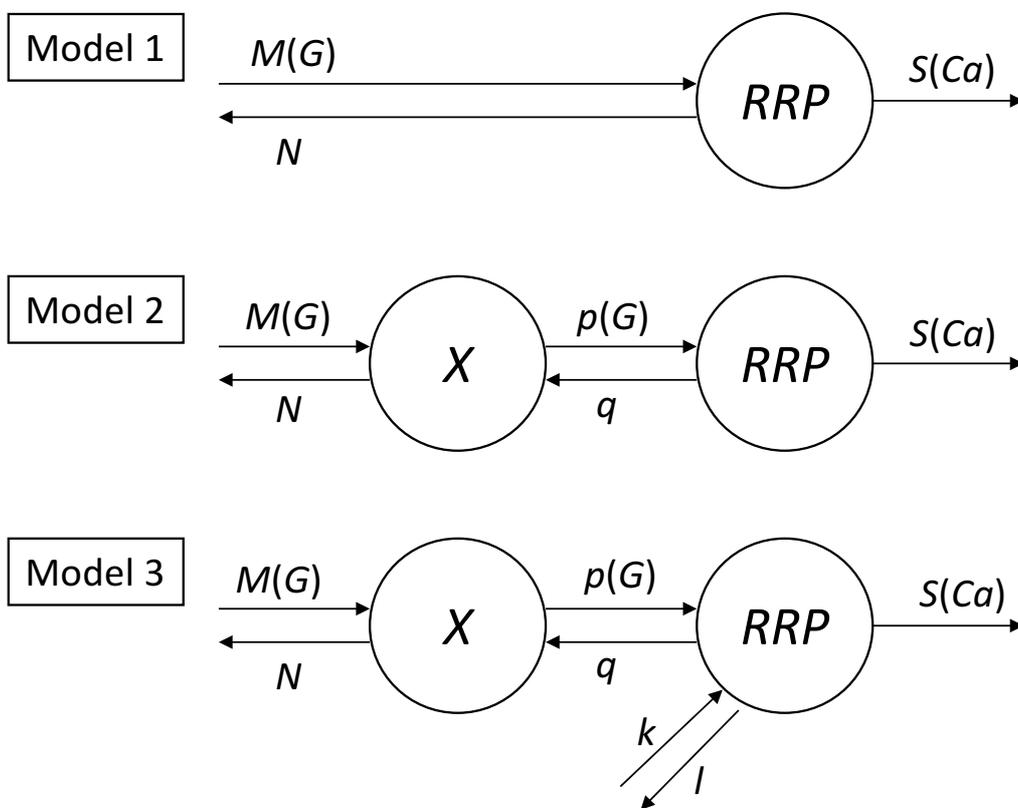
Figure 1
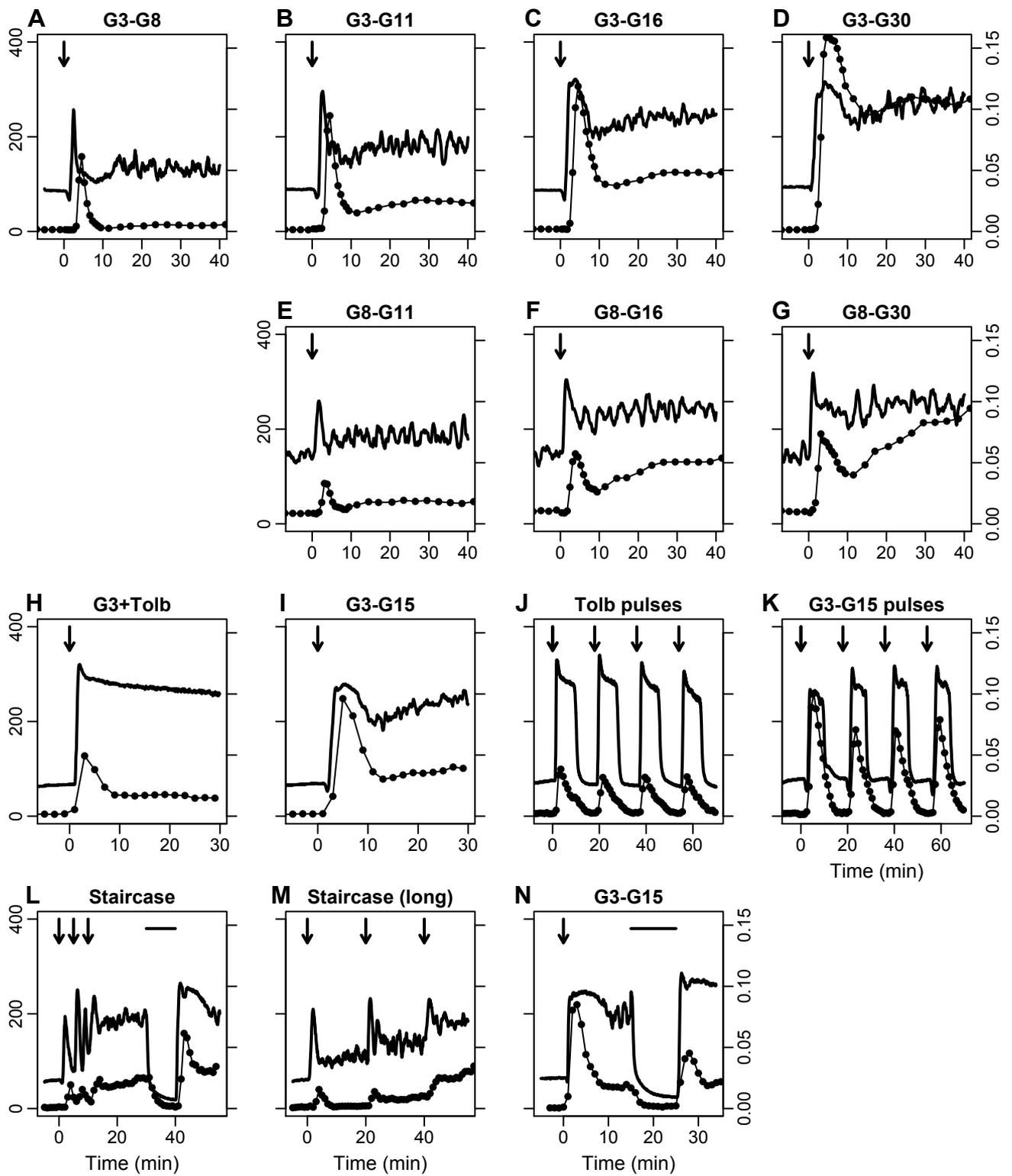
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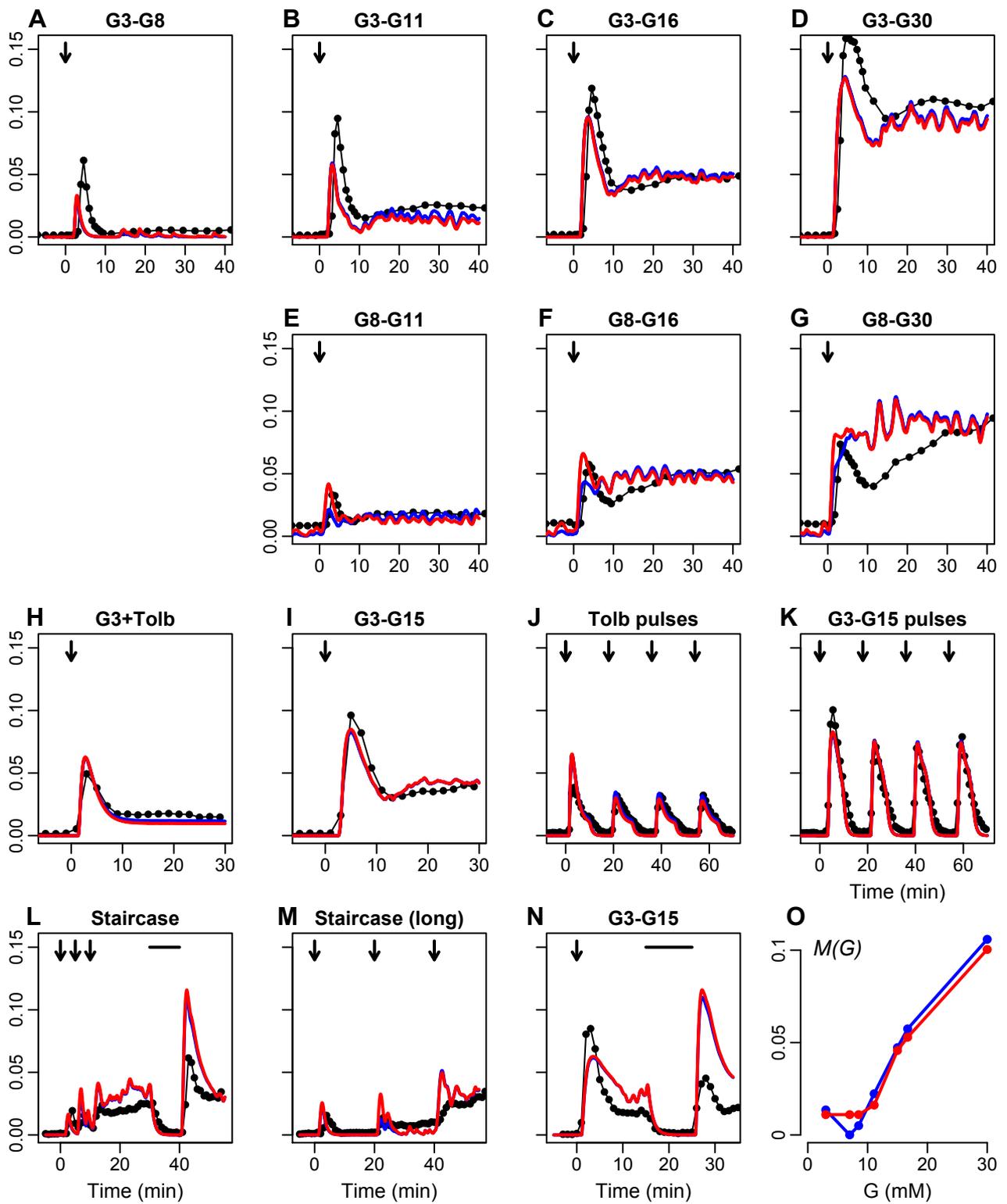
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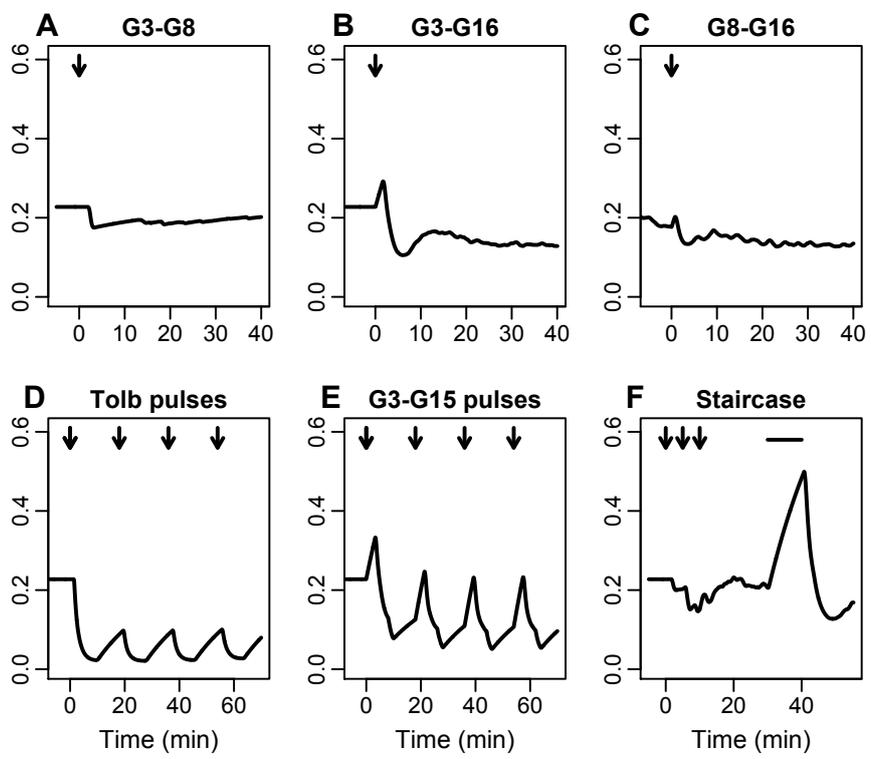
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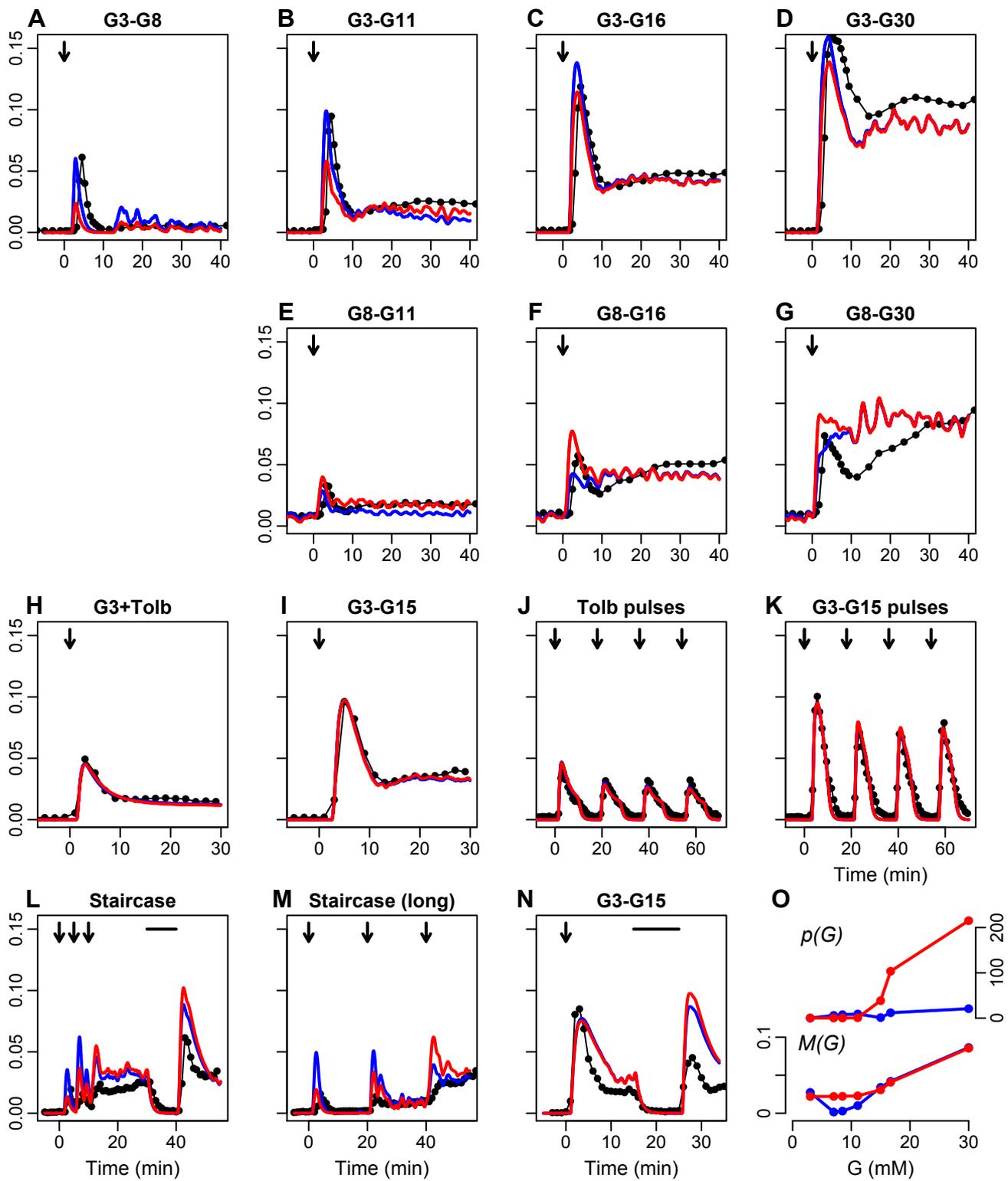
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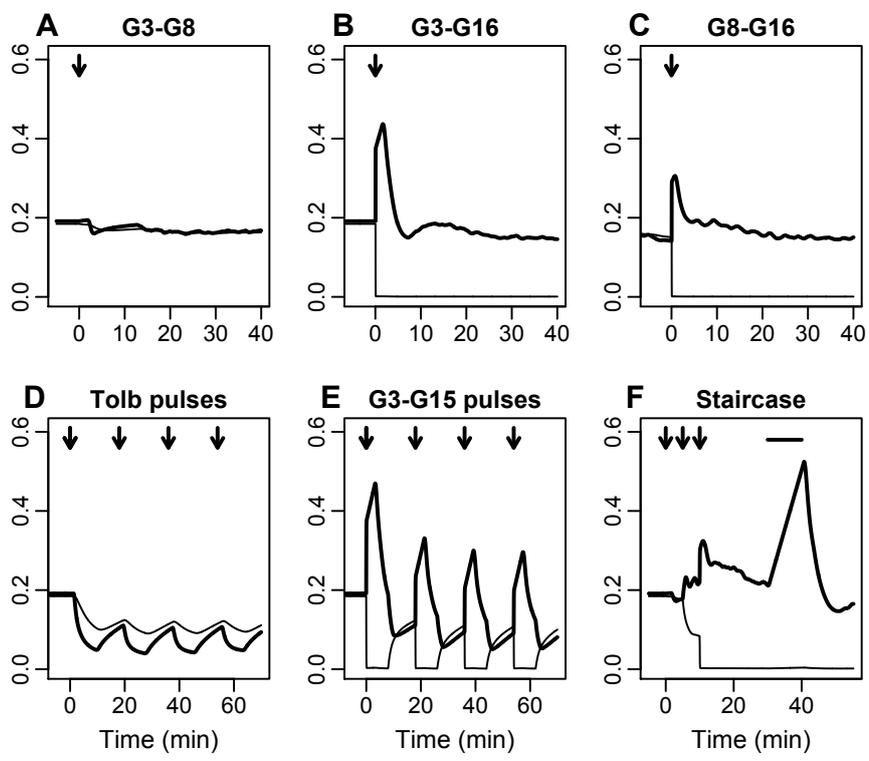
Figure 6

Figure 7

Figure 8