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1 #####
2 ## Title: A computational model for the thyroid axis in X. laevis larvae ##
3 ## ##
4 ## Script author: Jonathan T. Haselman ##
5 ## ##
6 ## Date created: 08-31-2022 ##
7 ## ##
8 ## Email: haselman.jon@epa.gov ##
9 ## ##
10 ## Citation: Haselman, J.T., Nichols, J.W., Mattingly, K.Z., Hornung, M.W. ##
11 ## and Degitz, S.J. 2023. A Biologically Based Computational ##
12 ## Model for the Hypothalamic-Pituitary-Thyroid (HPT) Axis in ##
13 ## Xenopus laevis larvae. Mathematical Biosciences (submitted) ##
14 ## ##
15 #####
16
17 install.packages("deSolve")
18 install.packages("gridExtra")
19 install.packages("ggplot2")
20 install.packages("ggplotify")
21 install.packages("plotly")
22 install.packages("data.table")
23 install.packages("Hmisc")
24 install.packages("xlsx")
25
26 library(deSolve)
27 library(gridExtra)
28 library(ggplot2)
29 library(ggplotify)
30 library(plotly)
31 library(data.table)
32 library(Hmisc)
33 library(xlsx)
34
35 ##### Load empirical control data from Haselman et al. (2020) #####
36
37 # Read csv into R - no file path necessary if located in working directory or project
38 # file
39 realdata <- read.csv(file="Data/Haselman_etal_2020_control_data.csv", header=TRUE, sep=
40 # ",")
41
42 # Convert data table into long form (i.e., all measurements in a single column with
43 # new column indicating previous column heading)
44 long.realdata <- melt(data.table(realdata), id.vars = 1:3, measure.vars=4:10)
45
46 # Remove NAs
47 na_vec <- which(!complete.cases(long.realdata))
48 long.realdata_no_na <- long.realdata[-na_vec,]
49
50 # Make a list storing a data frame for each analyte/endpoint as components
51 var.list <- lapply(by(long.realdata_no_na, long.realdata_no_na[, "variable"], identity),
52 # as.data.frame)
53
54 # Unique data frame for each variable
55 Wt_g <- var.list[[1]]
56 T3_bld_nM <- var.list[[5]]
57 T4_bld_nM <- var.list[[6]]
58 T4_glnd_pmol <- var.list[[4]]
59 MIT_glnd_pmol <- var.list[[2]]
60 DIT_glnd_pmol <- var.list[[3]]
61 FCN_real <- var.list[[7]]
62
63 ##### State variables and starting values for dynamic model #####
64
65 state <- c(

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64 BW = 0.28, # g; extrapolated to t=0
65 VMAXTSH = 0.325, # pmol/h; optimized
66 RKMTSH = 2E-8, # 1/h; optimized
67 KMTSH = 1.1E-6, # pmol/ml; 1/100th the starting CT3PLFF
68 ATSHPL = 0.00165, # pmole; optimized at t=0
69 FCNTOT = 8000, # follicular cell number; extrapolated to t=0
70 AIFC = 0.16, # pmole; starting amount of iodine in follicular cells; 20xCIPL
71 ATGTL = 0.4, # pmole; starting amount of Tg in lumen; exceeds amount of
iodinated Tg
72 AITGTL = 13.135, # pmole; 0.355 pmol Tg * 37 iodine atoms
73 AMITITL = 0.355, # pmole; 0.355 pmol Tg * 1 iodine atom
74 ADITITL = 4.26, # pmole; 0.355 pmol Tg * 12 iodine atoms
75 AT4ITL = 8.52, # pmole; 0.355 pmol Tg * 24 iodine atoms
76 AT4TOTPL = 0.0073, # pmole; extrapolated to t=0 and optimized
77 AT3TOTPL = 0.00112 # pmole; extrapolated to t=0 and optimized
78
79 )
80
81 ##### Parameter values for dynamic model #####
82
83 parameters<- c(
84
85 # Constants
86 RBW = 0.0027, # 1/h; rate of body growth
87 FCV = 1E-9, # ml; equivalent to 1 picoliter per cell
88 RFCNCONT = 40, # 1/h; rate of cell proliferation independent of TSH stimulation
89 RFCNMAX = 700, # 1/h; rate of cell proliferation as a result of TSH stimulation
90 KH = 0.6, # Hill constant
91 n = 3.5, # Hill coefficient - defines the slope of the relationship
92 CIPL = 1000, # pmol/ml; plasma iodine concentration
93 KDI = 1.1, # ml/h; iodine diffusion constant
94 KDT4 = 0.01, # unitless; dissociation constant; proportion of CT4TOTPL that is
CT4FFPL
95 KDT3 = 0.005, # unitless; dissociation constant; proportion of CT3TOTPL that is
CT3FFPL
96 KIFC = 4E4, # Wolff-Chaikoff inhibition constant set 2x starting CIFC
97 RRKMTSH = 1E-9, # 1/h; optimized
98 RVMAXTSH = 0.005, # pmol/h; optimized
99
100 # Clearance terms
101 CLT4CONJ = 4, # ml/h; to achieve t 1/2 value of about 2 hours
102 CLT3CONJ = 28.3, # ml/h; t 1/2 value of about
103 CLTSH = 0.11, # ml/h; t 1/2 value of about 0.37 h (21 min)
104
105 # Apparent volumes of distribution
106 AVDT4C = 0.156, # ml/g
107 AVDT3C = 0.186, # ml/g
108 AVDTSHC = 0.0554, # ml/g; approx. equal to plasma volume - very little if any TSH
enters tissues
109
110 # Km terms
111 KMNISTSH = 1, # pmol/ml; 10x starting CTSHPL
112 KMNIS = 1E5, # pmol/ml; 100x starting CIPL
113 KMTPO = 1, # pmol/ml; 10x starting CTSHPL
114 KMTGPO = 2E6, # pmol/ml; 100x starting CIFC
115 KMTGSYN = 1, # pmol/ml; 10x starting CTSHPL
116 KMTGPROT = 10, # pmol/ml; 100x starting CTSHPL
117 KMDIO2 = 0.167, # pmol/ml; 100x starting CT4FFPL
118 KMDIO3 = 0.011, # pmol/ml; 100x starting CT3FFPL
119
120 # Vmax terms
121 VMAXNISTSH = 2.55E7, # pmol/h/ml; optimized
122 VMAXTPOTSH = 4E7, # pmol/h/ml; optimized
123 VMAXTGSYN = 4400, # pmol/h/ml; optimized
124 VMAXTGPROT = 10000, # pmol/h/ml; optimized
125 VMAXDIO2 = 0.88, # pmol/h; optimized

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126     VMAXDIO3 = 0.58      # pmol/h; optimized
127
128 )
129
130 ##### Simulation time parameters for dynamic model #####
131
132 starttime <- 0
133 stoptime <- 240 # hours; 10 day experiment
134 dtout <- 0.1      # hours; integration time step
135 times <- seq(starttime, stoptime, dtout)
136
137 ##### Dynamic model #####
138
139 dynmodel <- function(t, state, parameters) {
140     with(as.list(c(state, parameters)), {
141
142         # Variable initializations
143         VFC = FCV*FCNTOT # ml/cell; 1 picoliter as determined by the gland morphometric study
144         CIFC = AIFC/VFC
145         AVDT4 = AVDT4C*BW
146         AVDT3 = AVDT3C*BW
147         CT4TOTPL = AT4TOTPL/AVDT4
148         CT3TOTPL = AT3TOTPL/AVDT3
149         CT4FFPL = CT4TOTPL*KDT4
150         CT3FFPL = CT3TOTPL*KDT3
151         AVDTSH = AVDTSHC*BW
152         CTSHPL = ATSHPL/AVDTSH
153
154         # Organism growth
155         dBW = RBW
156
157         # Plasma TSH
158
159         dVMAXTSH = RVMAXTSH
160
161         dRKMTSH = RRKMTSH
162
163         dKMTSH = RKMTSH
164
165         RTSHSEC = VMAXTSH/((CT3FFPL/KMTSH)+1)
166
167         RTSHM = CLTSH*BW*CTSHPL
168
169         RTSHPL = RTSHSEC-RTSHM
170
171         dATSHPL = RTSHPL
172
173         # Gland growth
174
175         FINDUC = 1/(1+(KH/CTSHPL)^n)
176
177         RFCNTSH = RFCNMAX*FINDUC
178
179         RFCNTOT = RFCNCONT+RFCNTSH
180
181         dFCNTOT = RFCNTOT
182
183         # Iodine transport into the follicular cells via NIS
184
185         VMAXNIS = (VMAXNISTSH*CTSHPL/(KMNISTSH+CTSHPL))*VFC
186
187         RNIS = VMAXNIS*CIPL/((KMNIS+CIPL)*(0.5+(CIFC/KIFC)))
188
189         # Iodine diffusion back to plasma from follicular cells
190
191         RIDIF = KDI*(CIFC-CIPL)*VFC

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192
193 # Thyroglobulin synthesis
194
195 RTGSYN = (VMAXTGSYN*CTSHPL/(KMTGSYN+CTSHPL))*VFC
196
197 # Iodine organification into Thyroglobulin
198
199 VMAXTPO = (VMAXTPOTSH*CTSHPL/(KMPOTSH+CTSHPL))*VFC
200
201 RTPO = (VMAXTPO*CIFC/(KMTPO+CIFC))*(1-(AITGTL/ATGTL)/37)
202
203 # Thyroglobulin proteolysis
204
205 RTGPROT = (VMAXTGPROT*CTSHPL/(KMTGPROT+CTSHPL))*VFC
206
207 # Iodine recycling from MIT/DIT resulting from TG proteolysis
208
209 RMITREC = RTGPROT*(AMITITL/ATGTL)
210
211 RDITREC = RTGPROT*(ADITITL/ATGTL)
212
213 # T4 secretion to plasma
214
215 RT4SEC = RTGPROT*((AT4ITL/ATGTL)/4)
216
217 # Collective rate of change of iodine in follicular cells
218
219 RIFC = RNIS-RIDIF-RTPO+RMITREC+RDITREC
220
221 dAIFC = RIFC
222
223 # Rate of change of thyroglobulin in the thyroid lumen
224
225 RTGTL = RTGSYN-RTGPROT
226
227 dATGTL = RTGTL
228
229 # Rate of change of organified iodine in thyroid lumen associated with Tg
230
231 RAITGTL = RTPO-(RTGPROT*(AITGTL/ATGTL))
232
233 dAITGTL = RAITGTL
234
235 # Rate of change of MIT in the thyroid lumen
236
237 RAMITITL = (RTPO*(1/37))-RMITREC
238
239 dAMITITL = RAMITITL
240
241 TG.MIT.pmoles = AMITITL/1
242
243 # Rate of change of DIT in the thyroid lumen
244
245 RADITITL = (RTPO*(12/37))-RDITREC
246
247 dADITITL = RADITITL
248
249 TG.DIT.pmoles = ADITITL/2
250
251 # Rate of change of T4 in the thyroid lumen
252
253 RAT4ITL = (RTPO*(24/37))-(RT4SEC*4)
254
255 dAT4ITL = RAT4ITL
256
257 TG.T4.pmoles = AT4ITL/4

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258
259 # Rate of change of T4 in the plasma
260
261 RT4DIO2 = VMAXDIO2*BW*CT4FFPL/(KMDIO2+CT4FFPL)
262
263 RT4CONJ = CLT4CONJ*BW*CT4FFPL
264
265 RAT4TOTPL = RT4SEC-RT4CONJ-RT4DIO2
266
267 dAT4TOTPL = RAT4TOTPL
268
269 # Rate of change of T3 in the plasma
270
271 RT3DIO3 = VMAXDIO3*BW*CT3FFPL/(KMDIO3+CT3FFPL)
272
273 RT3CONJ = CLT3CONJ*BW*CT3FFPL
274
275 RAT3TOTPL = RT4DIO2-RT3CONJ-RT3DIO3
276
277 dAT3TOTPL = RAT3TOTPL
278
279 # Key ratios
280
281 Io_ratio = CIFC/CIPL
282 MIT_DIT_ratio = TG.DIT.pmoles/TG.MIT.pmoles
283 MIT_T4_ratio = TG.T4.pmoles/TG.MIT.pmoles
284
285 # Output
286 list(
287   # Output state variables
288   c(dBW, dVMAXTSH, dRKMTSH, dKMTSH, dATSHPL, dFCNTOT, dAIFC, dATGTL,
289     dAITGTL, dAMITITL, dADITITL, dAT4ITL, dAT4TOTPL, dAT3TOTPL),
290   # Output amounts and concentrations
291   CIFC=CIFC, CIPL=CIPL, TG.MIT.pmoles=TG.MIT.pmoles, TG.DIT.pmoles=TG.DIT.pmoles,
292   TG.T4.pmoles=TG.T4.pmoles, CT4TOTPL=CT4TOTPL, CT4FFPL=CT4FFPL,
293   CT3TOTPL=CT3TOTPL, CT3FFPL=CT3FFPL, CTSHPL=CTSHPL,
294   # Output rates
295   RFCNTOT=RFCNTOT, RNIS=RNIS, RIDIF=RIDIF, RIFC=RIFC, RTPO=RTPO, RTGSYN=RTGSYN,
296   RTGPROT=RTGPROT, RTGTL=RTGTL, RAITGTL=RAITGTL, RAMITITL=RAMITITL, RADITITL=
297   RADITITL,
298   RAT4ITL=RAT4ITL, RMITREC=RMITREC, RDITREC=RDITREC, RT4SEC=RT4SEC,
299   RT4DIO2=RT4DIO2, RT4CONJ=RT4CONJ, RAT4TOTPL=RAT4TOTPL, RT3DIO3=RT3DIO3,
300   RT3CONJ=RT3CONJ, RAT3TOTPL=RAT3TOTPL, RTSHSEC=RTSHSEC, RTSHM=RTSHM,
301   # Output other terms
302   FINDUC=FINDUC, Io_ratio=Io_ratio, AVDT4=AVDT4, AVDT3=AVDT3, AVDTSH=AVDTSH)
303 }
304
305
306 ##### Run model #####
307 # Run model
308 out <- ode(y = state, times = times, func = dynmodel, parms = parameters, method = "rk4")
309
310 # Create data frame by hour
311 dynout <- as.data.frame(out[seq(from = 1, to = dim(out)[1], by = 1/dtout),])
312
313 ##### Generate graphs #####
314
315 # Plot modeled BW and control BW data
316 Body_weight <- ggplot()+
317   geom_smooth(data=dynout, aes(x=time, y=BW), color="black")+
318   geom_point(data=Wt_g, aes(x=Time_h, y=value))+
319   stat_summary(data=Wt_g, aes(x=Time_h, y=value), fun.data=mean_cl_normal) +
320   geom_smooth(data=Wt_g, aes(x=Time_h, y=value), method='lm', formula= y~x)+
321   ylab("Body weight (g)")
322 print(Body_weight)

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322
323 # Plot modeled concentration of total T4 in plasma and control data
324 T4_Plasma <- ggplot()+
325   geom_smooth(data=dynout, aes(x=time, y=CT4TOTPL), color="black")+
326   geom_point(data=T4_bld_nM, aes(x=Time_h, y=value))+
327   stat_summary(data=T4_bld_nM, aes(x=Time_h, y=value), fun.data=mean_cl_normal) +
328   geom_smooth(data=T4_bld_nM, aes(x=Time_h, y=value), method='lm', formula= y~x)+
329   ylab("Plasma T4 (nM)")
330
331 # Plot modeled concentration of total T3 in plasma and control data
332 T3_Plasma <- ggplot()+
333   geom_smooth(data=dynout, aes(x=time, y=CT3TOTPL), color="black")+
334   geom_point(data=T3_bld_nM, aes(x=Time_h, y=value))+
335   stat_summary(data=T3_bld_nM, aes(x=Time_h, y=value), fun.data=mean_cl_normal) +
336   geom_smooth(data=T3_bld_nM, aes(x=Time_h, y=value), method='lm', formula= y~x)+
337   ylab("Plasma T3 (nM)")
338
339 # Plot modeled concentration of MIT in thyroid lumen and control data
340 MIT_Lumen <- ggplot()+
341   geom_smooth(data=dynout, aes(x=time, y=TG.MIT.pmoles), color="black")+
342   geom_point(data=MIT_glnd_pmol, aes(x=Time_h, y=value))+
343   stat_summary(data=MIT_glnd_pmol, aes(x=Time_h, y=value), fun.data=mean_cl_normal) +
344   geom_smooth(data=MIT_glnd_pmol, aes(x=Time_h, y=value), method='lm', formula= y~poly(x
345   , 2))+
346   ylab("Amt MIT in lumen (pmoles)")
347
348 # Plot modeled concentration of DIT in thyroid lumen and control data
349 DIT_Lumen <- ggplot()+
350   geom_smooth(data=dynout, aes(x=time, y=TG.DIT.pmoles), color="black")+
351   geom_point(data=DIT_glnd_pmol, aes(x=Time_h, y=value))+
352   stat_summary(data=DIT_glnd_pmol, aes(x=Time_h, y=value), fun.data=mean_cl_normal) +
353   geom_smooth(data=DIT_glnd_pmol, aes(x=Time_h, y=value), method='lm', formula= y~poly(x
354   , 2))+
355   ylab("DIT in lumen (pmoles)")
356
357 # Plot modeled concentration of T4 in thyroid lumen and control data
358 T4_Lumen <- ggplot()+
359   geom_smooth(data=dynout, aes(x=time, y=TG.T4.pmoles), color="black")+
360   geom_point(data=T4_glnd_pmol, aes(x=Time_h, y=value))+
361   stat_summary(data=T4_glnd_pmol, aes(x=Time_h, y=value), fun.data=mean_cl_normal) +
362   geom_smooth(data=T4_glnd_pmol, aes(x=Time_h, y=value), method='lm', formula= y~poly(x,
363   2))+
364   ylab("Amt T4 in lumen (picomoles)")
365
366 # Plot modeled thyroid follicular cell proliferation and control data
367 Follicular_cells <- ggplot()+
368   geom_smooth(data=dynout, aes(x=time, y=FCNTOT), color="black")+
369   geom_point(data=FCN_real, aes(x=Time_h, y=value))+
370   stat_summary(data=FCN_real, aes(x=Time_h, y=value), fun.data=mean_cl_normal) +
371   geom_smooth(data=FCN_real, aes(x=Time_h, y=value), method='lm', formula= y~x)+
372   ylab("Follicular cell number")
373
374 SimsPlusData <- grid.arrange(MIT_Lumen, DIT_Lumen, T4_Lumen, T4_Plasma, T3_Plasma,
375   Follicular_cells, nrow=2)
376
377 print(SimsPlusData)
378
379 # Plot concentration of TSH in plasma
380 Plasma_TSH <- ggplot(dynout, aes(x=time, y=CTSHPL))+
381   geom_smooth(color="black")+
382   ylab("Plasma TSH (nM)")
383
384 # Plot follicular cell induction term
385 FC_Induction <- ggplot(dynout, aes(x=time, y=FINDUC))+
386   geom_smooth(color="black")+
387   ylab("Follicle cell induction term")
388

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385 # Plot total follicular cell proliferation rate
386 Total_FNC_rate <- ggplot(dynout, aes(x=time, y=RFCNTOT))+
387   geom_smooth(color="black")+
388   ylab("Total FCN prolif. rate")
389
390 # Set axis bounds for iodide ratio
391 y.max.Io_ratio <- 1.005*min(dynout$Io_ratio)
392 y.min.Io_ratio <- 0.995*min(dynout$Io_ratio)
393
394 # Plot iodide concentration factor between plasma and thyrocytes
395 Io_x_factor <- ggplot(dynout, aes(x=time, y=Io_ratio))+
396   geom_smooth(color="black")+
397   ylab("Ratio free Io CIFC/CIPL")
398
399 # Set axis bounds for CIFC
400 y.max.cifc <- 1.005*min(dynout$CIFC)
401 y.min.cifc <- 0.995*min(dynout$CIFC)
402
403 # Plot concentration of iodide in follicular cells
404 Io_FC <- ggplot(dynout, aes(x=time, y=CIFC))+
405   geom_smooth(color="black")+
406   ylab("Free Io follicular cells (nM)")
407
408 # Set bounds for Tg in thyroid lumen
409 y.max.ATGTL <- 1.005*min(dynout$ATGTL)
410 y.min.ATGTL <- 0.995*min(dynout$ATGTL)
411
412 # Plot amount of Tg in thyroid lumen
413 TG_Lumen <- ggplot(dynout, aes(x=time, y=ATGTL))+
414   geom_smooth(color="black")+
415   ylab("Amt TG in lumen (pmoles)")
416
417 # Plot concentration of free T4 in plasma
418 T4_Plasma_FF <- ggplot(dynout, aes(x=time, y=CT4FFPL))+
419   geom_smooth(color="black")+
420   ylab("Free T4 plasma (nM)")
421
422 # Plot concentration of free T3 in plasma
423 T3_Plasma_FF <- ggplot(dynout, aes(x=time, y=CT3FFPL))+
424   geom_smooth(color="black")+
425   ylab("Free T3 plasma (nM)")
426
427 SimsOther <- grid.arrange(Plasma_TSH, FC_Induction, Total_FNC_rate, Io_x_factor,
428   Io_FC, TG_Lumen, T4_Plasma_FF, T3_Plasma_FF, nrow=2)
429 print(SimsOther)
430
431 ##### Export data to excel #####
432 Data_out <- "Output/Dynamic model output.xlsx"
433
434 write.xlsx2(dynout, Data_out, sheetName="model_out", col.names=TRUE, row.names=FALSE,
append=TRUE)

```