SUPPLEMENTARY DATA

Sex chromosome complement contributes to sex differences in bradycardic baroreflex response

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Short title: Sex chromosomes in bradycardic sex differences

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EXPANDED METHODS

Genotyping
Tail samples were obtained from mice and genomic DNA was isolated using standard procedures. Genotypes were determined by PCR analysis by the presence of the Sry transgene [primers SryF (forward): CCT ACA GCC ACA TGA TA; SryR (reverse): GTC TTG CCT GTA TGT GAT GG] (1) and of the Y long-arm gene family Ssty [primers SstyF (forward): CTG GAG CTC TAC AGT GAT GA; SstyR (reverse): CAG TTA CCA ATC AAC ACA TCA C] (2). The autosomal gene Myogenin [primers MYOF (forward): TTA CGT CCA TCG TGG ACA GCA T; MYOR (reverse): TGG GCT GGG TGT TAG TCT TAT] served as an amplification control (3) yielding a 245-bp product in all genotypes. Amplification of DNA yielded the following products according to the genotypes: for XY-males the 384-bp Sry and the 302-bp Ssty; for XY-females the 302-bp Ssty; for XX-males the 384-bp Sry; while in XX-females only the myogenin control product was amplified.

Surgical procedures

Gonadectomy
Adult male and female mice were anesthetized with ketamine/xylazine mixture (ip, xylazine, 1 mg/kg, König and ketamine 162 mg/kg Holliday-Scott, Argentina). Bilateral incision was made in the scrotum region for male mice and just below the rib cage in the female mice in order to be able to perform bilateral gonadectomy. Afterwards, the muscle layer and the incision were sutured in place.

At the time gonadectomy was performed, no differences were reported in testosterone levels in XX-male vs. XY-male mice, or in XX-female vs. XY-female in SJL (3) and C57BL/6J (4) mice strain. Furthermore, gonadectomy resulted in undetectable levels of 17-β-estradiol (female) and testosterone (male) in MF1 mice of the FCG mouse model (5).

Chronic catheterization
Under ketamine/xylazine anesthesia, mice were surgically instrumented with intra-arterial catheters for direct measurement of arterial pressure and jugular vein catheters for drug administration. A middle incision was made in the neck and an end-heated 9 and 5 mm micro-renathane tubing (MRE25, Braintree Sci., Boston, MA) welded to a silastic catheter (Dow corning, 0.020 I.D. x 0.037 O.D.) was inserted into the left carotid artery and jugular vein respectively. The catheters were then firmly sutured in place, tunneled subcutaneously between the shoulder blades and connected to a stainless steel “elbow” made of 23-gauge hypodermic tubing. The cannulas were filled with sterile heparinized saline (50 U/mL) to prevent clotting and the external end of the elbow was closed up with a plastic cap (6).

REFERENCES
2. Turner JM, Mahadevaiah SK, Benavente R, Offenberg HH, Heyting C, Burgoyne PS. Analysis of male meiotic “sex body” proteins during XY


