ONLINE SUPPLEMENT

Cardiovascular Responses to Hypothalamic Arcuate Nucleus Stimulation in the Rat: Role of Sympathetic and Vagal Efferents

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Methods

Microinjections into the ARCN
The rats were placed in a prone position in a stereotaxic instrument with bite bar 11 mm below the interaural line. A hole (8-10 mm in diameter) was drilled in the midline at the junction of the two parietal bones caudal to the bregma. The microinjections were made through this hole, on either side of the midline, using multi-barreled glass-micropipettes (tip size 20–40 μm). The coordinates for the ARCN were: 3.6-3.8 mm caudal to the bregma, 0.1-0.2 mm lateral to the midline, and 9.8-10.2 mm deep from the dura; the same coordinates for the ARCN were used for all experiments unless indicated otherwise. The volume and duration of all microinjections into the ARCN were 50 nl and 5-10 s, respectively. Microinjections of artificial cerebrospinal fluid (aCSF, pH 7.4) into the ARCN were used as controls.

Intrathecal injection
The atlanto-occipital membrane was incised, the tip of a polyethylene tubing (PE-10), connected to a Hamilton microsyringe and filled with a drug or aCSF, was inserted under the dura mater and advanced caudally 6 cm to T9-T10 level. Combined solutions of the iGLUR antagonists (D-AP7 and NBQX) were injected intrathecally (volume 20 μl and duration 10 s). Intrathecal injections (20 μl) of aCSF were used as controls.

Application of drugs at T1-T4
The rats were placed in a prone position in a stereotaxic instrument. The dorsal surface of the spinal cord from C8 to T5 level was exposed by laminectomy and the dura was sectioned. The responses to ARCN stimulation were studied before and after the application of a tissue paper pledget soaked in combined iGLUR antagonist solution (20 μl) to the spinal cord surface at T1-T4. The spinal cord surface was irrigated with aCSF after the application of drugs and a fresh aCSF-soaked paper pledget was applied.

Nerve recording
The greater splanchnic and left renal nerves were exposed retroperitoneally in separate experiments, sectioned distally and whole nerve activity was recorded from the central end of each nerve by standard techniques. At the end of the experiment, the nerves were sectioned centrally and the remaining activity was considered to be the noise level.

Histology
The microinjection sites in the ARCN were marked by diluted India ink (50 nl) at the level 3.60-3.80 mm caudal to the bregma (n = 10). The animals were perfused and fixed with 4% paraformaldehyde, serial sections of the hypothalamus were cut (30 μm) and stained with cresyl violet. The microinjection sites were identified using a standard atlas.
Drugs and chemicals
The following drugs and chemicals were used: N-methyl-D-aspartic acid (NMDA), (+)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid hydrobromide (AMPA; non-NMDA receptor agonist), D(-)-2-amino-7-phosphono-heptanoic acid (D-AP7; NMDA receptor antagonist), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoxaline-7-sulfonamide disodium (NBQX disodium salt; non-NMDA receptor antagonist), muscimol (GABA<sub>A</sub> receptor agonist), L-glutamate monosodium (L-Glu), L-phenylephrine hydrochloride (PE), isoflurane, and urethane. All of the solutions for the microinjections and intrathecal injections were freshly prepared in aCSF (298 ± 2 mOsmol/kg; pH 7.4). Where applicable, the concentration of drugs refers to their salts. The sources of different drugs and chemicals were as follows: AMPA and D-AP7 (Tocris Cookson Inc., Ellisville, MO, USA) and isoflurane (Baxter Pharmaceutical Products, Deerfield, IL, USA). All other drugs and chemicals were obtained from Sigma Chemicals (St. Louis, MO, USA).

Statistical analyses
Mean and standard error of mean (SEM) were calculated for maximum changes in mean arterial pressure (MAP) and HR. Comparisons of maximum changes in MAP and HR in different groups of rats were made by one-way analysis of variance (ANOVA) followed by Tukey-Kramer’s multiple comparison. In experiments testing for tachyphylaxis and effect of spinal cord iGLUR block or combined vagotomy and spinal cord iGLUR block, comparisons of the maximum changes in MAP and HR elicited by microinjection of NMDA into the ARCN were made by repeated measures ANOVA followed by Tukey-Kramer’s multiple comparison test. Student’s paired t-test was used in all other statistical analyses. For the analysis of nerve activity, control value represented the average amplitude of integrated greater splanchnic nerve activity (GSNA) and renal nerve activity (RSNA) during 35 sec period before the i.v. administration of PE or the microinjections of drugs into the ARCN. Maximum change in GSNA and RSNA amplitude, induced by i.v. administration of PE or microinjections of drugs into the ARCN, was expressed as percent change from the control value of GSNA and RSNA amplitude. The mean values of the integrated nerve signals were compared using Student's paired t-test. In all cases, the differences were considered significant at P < 0.05.

References