Effect of age on cardiac norepinephrine release in the female rat

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ABSTRACT. We previously demonstrated an age-related decline in K+-induced norepinephrine (NE) release from cardiac synaptosomes prepared from 6- and 24-month-old male F344 rats. The purpose of the present study was to determine if the age-related decrease in NE release seen in male F344 rats is also present in female F344 rats. K+-induced NE release was assessed in cardiac synaptosomes prepared from 6-, 12-, 18-, and 24-month-old male and female F344 rats. NE release was significantly greater in young male rats, compared to old male rats. However, no age-related decrease in NE release was observed in the female rats. In contrast to previous observations in male rats, raising extracellular [Mg2+], an inorganic Ca2+ channel blocker, reduced NE release to the same extent in all female ages. Omega-conotoxin, an organic Ca2+ channel blocker, also decreased NE release to the same extent in all female ages. These studies suggest that in contrast to aging male rats, cardiac adrenergic nerve terminals of aging female rats maintain their capacity to release NE.

INTRODUCTION

We have used cardiac synaptosome preparations developed in our laboratory to investigate the role of Ca2+ movement in the age-related reduction in norepinephrine (NE) release from cardiac adrenergic nerve terminals (1, 2). Synaptosomes are resealed presynaptic nerve endings (3). NE release from synaptosomes is Ca2+-dependent (4, 5) and can be inhibited by omega-conotoxin which binds to and blocks neuronal N-type Ca2+ channels (6, 7). We previously demonstrated an age-related decline in K+-induced NE release from cardiac synaptosomes prepared from 6- and 24-month-old male F344 rats (2, 8). The age-related reduction in NE release induced by K+, coupled with the observation that the capacity of ionomycin, a calcium ionophore, to induce NE release is similar in young and old cardiac synaptosomes, points to a reduction in Ca2+ entry during depolarization. Therefore, the age-related decline in NE release is due, in part, to a defect in Ca2+ movement through neuronal Ca2+ channels, rather than an alteration in Ca2+ activation of intracellular events leading to NE release. Roberts (8) also showed that raising extracellular [Mg2+], an inorganic Ca2+ channel blocker, reduces NE release, and that old male rats exhibited a greater reduction in NE release to small increases in [Mg2+]. Omega-conotoxin, an organic Ca2+ channel blocker, decreased release; the percent reduction in release was greater in young male rats. These experiments suggest that cardiac adrenergic nerve terminals of young male rats may have spare Ca2+ channels which require higher concentrations of Mg2+ to inhibit, and suggest that cardiac adrenergic nerve terminals of young male rats may have more omega-conotoxin sensitive Ca2+ channels compared to old male rats.

Experiments performed with isolated heart preparations obtained from male F344 rats also demonstrated that cardiac NE release induced by either electrical stimulation or K+ is reduced in aging male rats (9-11). However, Turner (12) reported that electrically-induced NE release from isolated hearts obtained from female F344 rats was similar at 6 and 24 months of age. This suggests that in the female rat heart there is no age-related reduction in Ca2+ movement into the cardiac adrenergic nerve terminals. The purpose of the present study was to determine if the age-related decrease in NE release seen in cardiac synaptosomes prepared from male F344 rats is also present in cardiac synaptosomes prepared from female F344 rats.
MATERIALS AND METHODS

Animals

Male and female Fischer 344 rats at 6, 12, 18, and 24 months of age were used in the present study. These ages were selected to elucidate changes specific to aging, while avoiding the developmental changes in rats younger than 6 months and the disease-related changes in rats older than 24 months. Rats were maintained under barrier conditions at Harlan Laboratories, Inc. (Indianapolis, IN). In our institution, male and female rats were housed in separate rooms, under barrier conditions in standard filtered cages, two rats per cage, in a temperature-regulated environment (21±1°C) with a 12-hour light/dark cycle. The animals were fed ad libitum with a pasteurized rodent diet (20% protein, 5% fat) and autoclaved water adjusted to pH 3.

Preparation of synaptosomes

The method developed in this laboratory for obtaining cardiac synaptosomes has been described by Aloyo (1) and Snyder (2). Rats were killed by decapitation to avoid the effects of anesthetic agents on uptake and release of NE. The hearts were then weighed and individually minced in 0.32 M sucrose containing 1 mM EGTA. The minced heart tissue was digested with 44 mg collagenase (class II, Worthington Biochemicals) per 10 mL HEPES-buffered saline solution (HBS) per gram wet heart weight for 40 minutes at 37° C. HBS (pH 7.4 at 22° C) contained 50 mM HEPES, 144 mM NaCl, 5 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂ and 10 mM glucose. The buffer also contained 1 mM pargyline to inhibit enzymatic destruction of NE, and 1 mM ascorbic acid to prevent oxidation of NE. The buffer was oxygenated prior to and during digestion. After homogenization and centrifugation in 0.32 M sucrose, the final pellet (P2) was resuspended in 3 mL ice-cold HBS. The P2 pellet contains the cardiac synaptosomes (1).

A 5 µL aliquot of the resuspended P2 was used for protein determination by the Bradford method (13).

Superfusion and ³H-NE release from cardiac synaptosomes

The P2 preparation was diluted to 1 mg protein/mL of ice-cold HBS, and then incubated with 300 nM ³H-NE (42 Ci/mmole, NEN) for 1 hour at 37°C. 200 µL aliquots of the ³H-NE/P2 preparation were loaded into 300 µL chambers of a Brandel 12-chamber superfusion system (Biomedical Research and Development Laboratories, Inc., Gaithersburg, MD). Whatman GF/B filters retained the synaptosome preparation in the chambers. HBS with 0.2% bovine serum albumin was perfused through the system at a rate of 250 µL/min. The buffer reservoir was oxygenated. After perfusion for 45 minutes, effluent from the chambers was collected in 5-minute fractions (1.25 mL/fraction), and the basal release of ³H-NE was determined over 30 minutes.

The cardiac synaptosomes in the superfusion system were stimulated by perfusing for 2 minutes with a buffer containing high concentrations of K⁺. The high K⁺ buffers mix with the normal buffer, and are diluted to 30% of their original concentration before reaching the chambers. For example, under these experimental conditions, buffer containing 150 mM K⁺ reaches a peak concentration in the chambers of only 45 mM K⁺. This peak concentration of K⁺ which depolarized the synaptosomes in each chamber is referred to rather than the actual K⁺ concentration in the depolarizing buffer.

Since the preparation of the 150 mM K⁺ depolarizing buffer would require the removal of all of the Na⁺ (144 mM) plus an additional 6 mM of another ion to remain isotonic with the normal perfusion buffer, the high K⁺ depolarizing buffers were made by adding additional KCl to the normal buffer without removing NaCl. This approach was taken in order not to reduce the concentration of ions that may influence NE release. Experiments were performed with hypertonic depolarizing buffers in which additional NaCl was added instead of the additional KCl. These buffers did not induce ³H-NE release. The Mg²⁺ buffers were also made by adding additional MgCl₂ without altering the concentration of Ca²⁺. In experiments testing the effect of Mg²⁺ on NE release, any change in the [Ca²⁺] to compensate for the changes in [Mg²⁺] would also affect NE release.

To terminate an experiment, the synaptosomes are perfused with water; this results in the release of the remaining ³H-NE into the last fractions collected. The ³H-NE released by rupturing the synaptosomes was used in calculating the fractional release of ³H-NE collected in previous fractions (see below). All fractions were counted in a Beckman scintillation counter using 3 mL of Liquiscint scintillation fluid.

The ³H-NE collected in each fraction was expressed as a percentage of the ³H-NE in the chamber at the time the fraction was collected. This percentage is the fractional release. Because it is a percentage, fractional release is independent of the total dpm present in any experiment. Fractional release was calculated for each fraction by using the following formula:

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\text{% Fractional Release from Fraction 'A' = } \frac{(\text{DPM of Fraction 'A') x 100}}{(\text{Total DPM Collected) - (DPM Collected Prior to Fraction 'A')})}
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