HYPERTENSION Supplement

Dipeptidyl Peptidase IV in Angiotensin-Converting Enzyme Inhibitor-Associated Angioedema

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Methods

To account for the possibility that endogenous bradykinin was increased in cases compared to controls, we drew blood for measurement of endogenous bradykinin and DPPIV antigen and activity in an additional 6 cases (3 during acute angioedema, 3 during the convalescent phase) and 17 ACE inhibitor-exposed controls.

Laboratory analysis

APP activity was measured using a quenched fluorimetric substrate that mimics the structure of des-Arg⁹-bradykinin and is specific for mAPP.¹ APN activity was determined by incubating sera with ala-p-nitroanilide at 37°C in an adaptation of a method previously described.²

The degradation half-lives of bradykinin, des-Arg⁹-bradykinin, and substance P were determined after the addition of known concentrations of exogenous bradykinin or substance P to serum at 37°C.³ The reaction was stopped after various incubation periods by adding cold anhydrous ethanol at a final concentration of 80% vol/vol. Samples were then incubated at 4°C for 1 h and centrifuged (4°C, 15 min, 3000g). The supernatant was decanted and evaporated to dryness and the residues were stored at 20°C until quantification of peptides. Bradykinin and des-Arg⁹-bradykinin were assayed using specific competitive chemiluminescent enzyme immunoassays, as previously described.⁴ ⁵
For the measurement of substance P, the evaporated residue was reconstituted in 200 μL of 5 mM KH₂PO₄, pH 3.0, 25% acetonitrile with 1.0% H₃PO₄. Substance P was separated from its metabolites using an Agilent 1100 Series system (Agilent Technologies Canada, Mississauga, Ca) with a 2-sulfoethyl aspartamide column (PolySULFOETHYL A TM, The Nest Group, Inc, Southboro, MA) and a linear gradient of KCl (0-300 mM) in 5 mM KH₂PO₄, 25% acetonitrile (vol/vol), pH 3.0 for 30 min. The retention time of native substance P was 24.5 min and its detection was obtained at 214 nm. The kinin or substance P (S) hydrolysis rate constant (k) was obtained with a first-order equation S = S₀ x e⁻ᵏᵗ. The degradation half life, t₁/₂, was calculated as t₁/₂ = ln(2)/k.

Blood for measurement of endogenous bradykinin was drawn into cold anhydrous ethanol at a final concentration of 80% vol/vol. The samples were then incubated for 1 hour at 4°C and centrifuged (2000 x g, 15 min, 4°C) to allow the complete precipitation of the precursors of kinins. The supernatant was saved at -70°C until the time of assay. Collected supernatant was decanted and evaporated to dryness under nitrogen at 37°C and reconstituted in 1.0 ml of distilled water. The pH of the solution was adjusted to 2-3 with 1.0 N HCL and the sample was washed twice with 3.0 ml of diethyl ether to remove lipids. After removal of diethyl ether by aspiration, the sample pH was adjusted to 6-7 with 1.0 N NaOH. Samples were then evaporated to dryness under nitrogen at 37°C and reconstituted in 75ul of assay buffer on the day of assay. Bradykinin concentrations were determined by use of a commercially available enzyme immunoassay (Bachem [Peninsula Laboratories], San Carlos, Calif). The minimal detectable concentration for
this kit is 0.02-0.04 ng/ml, with a range of detection of 0-10 ng/ml. Intra-assay variation is <5%, while inter-assay variation <14%. The antibodies in this kit display 100% cross-reactivity with several bradykinin-related substances, including lys-bradykinin, biotinyl-bradykinin, (Tyr\(^0\))-bradykinin, des-Arg\(^1\)-bradykinin, and bradykinin-(4-9). The antibodies partially cross-react (40%) with bradykinin-(5-9).

Results

Relationship between DPPIV and endogenous bradykinin

Endogenous bradykinin concentrations were similar to the normal range reported by Nussberger et al.\(^6\) Bradykinin concentrations were not significantly elevated in cases compared to controls (Figure S1). In addition, there was no correlation between bradykinin concentrations and either DPPIV antigen (\(r^2 = 0.06, P = 0.26\)) or activity (\(r^2 = 0.04, P = 0.38\)).
References


Figure legend.

Figure S1. Endogenous bradykinin concentrations in individuals with angiotensin-converting enzyme inhibitor-associated angioedema and angiotensin-converting enzyme inhibitor-exposed controls.
Figure S1.