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-Arg$^9$-bradykinin and is specific for mAPP.[1] APN activity was determined by incubating sera with ala-p-nitroanilide at 37$^o$C in an adaptation of a method previously described.[2]

The degradation half-lives of bradykinin, des-Arg$^9$-bradykinin, and substance P were determined after the addition of known concentrations of exogenous bradykinin or substance P to serum at 37ºC.[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 4ºC for 1 h and centrifuged (4ºC, 15 min, 3000g). The supernatant was decanted and evaporated to dryness and the residues were stored at 20ºC until quantification of peptides. Bradykinin and des-Arg$^9$-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 $KH_2PO_4$, pH 3.0, 25% acetonitrile with 1.0% $H_3PO_4$. 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 $KH_2PO_4$, 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 $S = So \times e^{-kt}$. The degradation half life, $t_{1/2}$, was calculated as $t_{1/2} = \ln(2) / k$.

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 4ºC and centrifuged (2000 x *g*, 15 min, 4ºC) 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


1. Molinaro G, Carmona AK, Juliano MA, Juliano L, Malitskaya E, Yessine MA, Chagnon M, Lepage Y, Simmons WH, Boileau G, Adam A. Human recombinant membrane-bound aminopeptidase P: production of a soluble form and characterization using novel, internally quenched fluorescent substrates. *Biochem J.* 2005; 385:389-397.

2. Lendeckel U, Wex T, Reinhold D, Kahne T, Frank K, Faust J, Neubert K, Ansorge S. Induction of the membrane alanyl aminopeptidase gene and surface expression in human T-cells by mitogenic activation. *Biochem J.* 1996; 319 ( Pt 3):817-821.

3. Cyr M, Lepage Y, Blais C, Jr., Gervais N, Cugno M, Rouleau JL, Adam A. Bradykinin and des-Arg(9)-bradykinin metabolic pathways and kinetics of activation of human plasma. *Am J Physiol Heart Circ Physiol.* 2001; 281:H275-H283.

4. Decarie A, Drapeau G, Closset J, Couture R, Adam A. Development of digoxigenin-labeled peptide: application to chemiluminoenzyme immunoassay of bradykinin in inflamed tissues. *Peptides.* 1994; 15:511-518.

5. Raymond P, Drapeau G, Raut R, Audet R, Marceau F, Ong H, Adam A. Quantification of des-Arg9-bradykinin using a chemiluminescence enzyme immunoassay: application to its kinetic profile during plasma activation. *J Immunol Methods.* 1995; 180:247-257.

6. Nussberger J, Cugno M, Amstutz C, Cicardi M, Pellacani A, Agostoni A. Plasma bradykinin in angio-oedema. *Lancet.* 1998; 351:1693-1697.

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.