Aminopeptidase A is Angiotensinase A

I. Quantitative Histochemical Studies in the Kidney Glomerulus *

P. Kugler

Institute of Anatomy, University of Würzburg (Chair: Prof. Dr. T.H. Schiebler), Koellikerstraße 6, D-8700 Würzburg, Federal Republic of Germany

Summary. Quantitative histochemical measurements of aminopeptidase A (APA; E.C.3.4.11.7) were done kinetically in the kidney glomeruli of rat and mouse with an instrumental setup consisting of a microdensitometer and a computer-supported morphometric system. The histochemical demonstration of APA was carried out using the simultaneous azo coupling technique (purest-grade Fast Blue B as coupling agent and \(\alpha\)-L-glutamic acid-4-methoxy-2-naphthylamide as substrate). The methodological studies show that APA activity is calcium-ion-dependent and increases linearly with the thickness of the tissue section (3–12 \(\mu\)m) and that the time-course of APA activity as determined by linear regression is linear only for the first 1 to 2 min of the reaction. – Kinetic measurements indicate a 40% decrease in APA activities when \(\alpha\)-L-glutamic acid-4-methoxy-2-naphthylamide (\(\alpha\)-L-Glu-MNA) is replaced by \(\alpha\)-L-aspartic acid-4-methoxy-2-naphthylamide. When \(\alpha\)-L-Glu-MNA is replaced with 5-alanine-4-methoxy-2-naphthylamide, which is a substrate of aminopeptidase M (APM) only very low reaction rates are measurable (about 1.4% of those with \(\alpha\)-L-Glu-MNA). 100 and 130 mM NaCl in the incubation medium increase APA activities by approximately 16%–17%. – To clarify the functional importance of APA in the kidney, their activities were measured under the influence of angiotensins. The glomerulus was selected as the measuring site, for besides APA it contains no APM or other peptidases that could degrade angiotensins (the glomerular dipeptidyl peptidase IV is not inhibited by angiotensin II). Using the Lineweaver-Burk plot, we determined a \(K_m\) of 0.16 mM for the APA in rat glomeruli and 0.14 mM in mouse glomeruli. The \(V_{max}\) in mouse glomeruli is 1.6 times higher than in rat glomeruli. Angiotensin I, II and III competitively inhibit APA in the rat and mouse glomeruli. – With quantitative histochemical techniques it was possible to show that APA is equivalent to angiotensinase A (splitting off the N-terminal aspartic acid from angiotensin I and II).

* Supported by the Deutsche Forschungsgemeinschaft (SFB 105)
Introduction

The octapeptide angiotensin II (ANG II) is the key effector substance of the renin-angiotensin system (Werning 1972; Page and Bumpus 1974; Hierholzer 1977; Vecsei et al. 1978; Johnson and Anderson 1980 lit.). It has a very short half-life in the body equal to about one circulation time (Hodge et al. 1967). According to previous studies, this means a rapid deactivation of the peptide hormone by enzymatic degradation (Ryan 1974 lit.). The enzymes that degrade the angiotensins are called angiotensinases (E.C.3.4.99.3). Angiotensin degradation takes place mainly in the various body tissues (Hodge et al. 1967). One of the most important of these sites is the kidney (Hodge et al. 1967; Leary and Ledingham 1969; Carone et al. 1980). It is known from biochemical studies that different enzymes are available in the kidney for the degradation of angiotensins: aminopeptidase A (Glenner et al. 1962), called also angiotensinase A (Nagatsu et al. 1965; Khairallah and Page 1967; Nagatsu et al. 1970), angiotensinase B (Regoli et al. 1963; Matsunaga 1971) and angiotensinase C (Yang et al. 1968; Matsunaga 1971). So far the only one of the foregoing peptidases that has been histochemically localized is aminopeptidase A (APA; E.C.3.4.11.7; Glenner and Folk 1961; Glenner et al. 1962; Lojda and Gossrau 1980; Kugler 1981b), which splits z-L-glutamic acid-2-naphthylamide (z-L-Glu-2NA). This membrane-bound calcium-dependent enzyme was found by light microscopy in the glomerulus and the proximal tubule of the kidney (Glenner et al. 1962; Lojda and Gossrau 1980; Kugler 1981b) and by ultracytochemistry in the endothelial cells and podocytes of the glomerulus (Kugler 1982). While it could be shown biochemically that the z-L-Glu-2NA-splitting enzyme is an angiotensinase (Hess 1965; Nagatsu et al. 1970) which splits off the N-terminal amino acid L-aspartic acid from ANG I or II, it could not be proved that the histochemically localized APA functions as an angiotensinase.

In the present study, we have developed quantitative histochemical methods to determine: 1. the ion dependency of APA (cf. Glenner and Folk 1961; Glenner et al. 1962; Hess 1965; Nagatsu et al. 1970; McDonald and Schwabe 1979; Kenny 1979; Lojda and Gossrau 1980; Kugler 1981b) and 2. the influence of different angiotensins on APA to realize the significance of the histochemically demonstrable APA in angiotensin degradation. The kidney glomerulus of rat and mouse was selected as the measuring site, for it contains no aminopeptidase M (Wachsmuth 1968; Wachsmuth and Torhorst 1974; Wachsmuth and Donner 1976; Kugler 1981b) which could interact with the APA.

Materials and Methods

The investigations were performed on 50 male Wistar rats and 30 male NMRI mice of our own breeding (random-bred closed colony). The animals were housed in Makrolon cages (4 rats per cage, 4-6 mice per cage) at 21 ± 2°C with a 12-h light-dark cycle (light from 7.00 a.m. to 7.00 p.m., dark from 7.00 p.m. to 7.00 a.m.) and were given tap water and standard Altromin diet (TPF 1320, pelleted) ad libitum. The animals were selected according to age and weight (rats and mice 75-95 days old; rats 280-300 g, mice 35-45 g). Rats and mice were decapitated under light ether anesthesia between 11.00 a.m. and 12.00 noon, the kidneys were quickly removed, and pieces of the organ were placed on specimen holders, wrapped in transparent film and frozen in N2-cooled propane (Winckler 1970). Sections were prepared in a cryostat (System Dittes-Duspiva) at −25°C to −30°C C.