Upsala J Med Sci 93: 297–299, 1988

Renal Handling of Bovine I^{125}-superoxide Dismutase in the Avian Kidney

Short Communication

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Superoxide dismutase (SOD) is an enzyme that in normal mammalian tissues catalyzes the dismutation reaction of superoxide anion ("scavenging" of oxygen free radicals) (1). It has been unequivocally shown in experimental studies that pretreatment with SOD will reduce tissue damage in the kidneys and the heart following reperfusion after a "now-flow" situation in these organs (2,3). The enzyme, which has a molecular weight of around 31,200 is, however, rapidly eliminated from the circulation, mainly by the kidneys (4). We have studied the renal handling of 125-I-SOD by use of the modified Sperber technique which differentiates between peritubular and luminal uptake of substances, e.g. peptides, in the avian kidney (5).

The avian kidney has a renal portal system, i.e. venous blood from the hindleg perfuses peritubular sinuses in the ipsilateral kidney before entering the systemic circulation. Portal blood reaches the peritubular side of both proximal and distal tubular cells during first passage through the sinuses. The glomeruli, on the other hand, are bypassed during first passage and are reached by the venous blood from the leg only after recirculation, i.e. through the arterial circulation when both kidneys are perfused simultaneously and equally. Thus, the renal portal system allows the investigator to create an asymmetry between the kidneys. Portal injection of a substance that binds to the peritubular side of tubular cells will result in more substance being bound in the injected kidney than in the contralateral kidney due to greater quantity of substance during first passage through the peritubular sinuses. A substance, that is subject solely to luminal reabsorption, subsequent to glomerular filtration, on the other hand, will accumulate equally in the two kidneys since filtration occurs only after recirculation of the substance, i.e.

under equal conditions for the two kidneys. For details about the method, see Odlind (6), Milton and Odlind (5).

Following portal injection of a mixture of bovine 125-I-SOD (supplied by Pharmacia, Uppsala, Sweden) and the extracellular marker 51-Cr-EDTA (Behringwerke AG, Marburg, FRG), with an activity ratio (125-I/51-Cr) in the injection solution of 1.0, hens were killed at 1 or 10 min after injection. Isoptope activity in the kidneys was determined in a two-channel gamma spectrometer (Packard Instrument Co., USA). Appropriate corrections were made for crossover to obtain net activity.

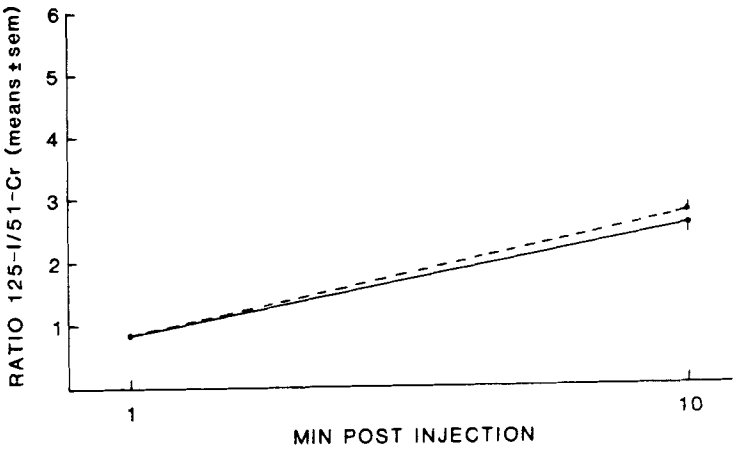

Figure 1: 125-I/51-Cr ratio in injected (●——●) and control (O---O) kidneys after renal portal injection of 125-I-superoxide dismutase (SOD) and 51-Cr-EDTA (means ± SEM). Number of animals; 3 at each time.

At 1 min after the injection 125-I-SOD was equally distributed in the two kidneys (Fig 1). This clearly shows that the bovine enzyme is not taken up by, or bound to proximal or distal tubular cells from the peritubular side in the avian kidney. This then in contrast to e.g. insulin that is extracted from peritubular blood and to some extent taken up into proximal tubular cells (7). It has been shown earlier with this model that the kidney content of 51-Cr-EDTA is similar at 1 and 10 min after injection in both kidneys and that there is no accumulation of the free iodine label (5). Thus, the distinct and parallel increase of the iodine to chromium activity ratios from 1 to 10 min after the injection (Fig 1) shows 125-I-SOD to be accumulated similarly in both kidneys since the denominator of the ratio did not change. This demonstrates that 125-I-SOD was reabsorbed into or bound to tubu-

lar cells from the luminal side of the avian nephron i.e. subsequent to glomerular filtration of the enzyme. This is compatible with what would be expected from the renal handling of a neutral protein with a molecular weight around 30,000 (8). Further studies, e.g. using autoradiography, are needed to determine the cellular localization of the renal uptake/binding of SOD and where along the nephron that this occurs.

REFERENCES

1. Fridovich, I.: The biology of oxygen radicals. Science 201:875-888, 1978.
2. Bayati, A., Hellberg, O., Odlind, B. & Wolgast, M.: Prevention of ischemic acute renal failure with superoxide dismutase and sucrose. Acta Physiol Scand 130:367-372, 1987.
3. Jolly, S.R., Kane, W.J., Baike, M.B., Abrams, G.D. & Lucchesi, B.R.: Canine myocardial reperfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. Circ Res 54:277-285, 1984.
4. Odlind, B., Appelgren, L.-E., Bayati, A. & Wolgast, M.: The tissue distribution of I^{125} labelled bovine superoxide dismutase (SOD) in the rat. Pharmacol Toxicol 62:95-100, 1988.
5. Milton, A. & Odlind, B.: Renal tubular accumulation of organic substances: a new in vivo method which differentiates between luminal and peritubular uptake. Acta Physiol Scand 123:237-248, 1985.
6. Odlind, B.: A modified Sperber technique for direct estimation of true renal tubular excretion fraction. Acta Physiol Scand 103:404-412, 1978.
7. Milton, A., Hellfritzsch, M. & Christensen, E.I.: Basolateral binding and uptake of I^{125} insulin in proximal tubular cells after peritubular extraction in the avian kidney. Diabetes Res 7:189-195, 1988.
8. Baumann, K.: Renal transport of proteins. In: Renal Transport of Organic Substances. Editors: Greger R, Lang F, Silbernagl S. Berlin, Springer Verlag, 118-133, 1981.

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