our findings are consistent with the concept of a 'dysautonomia of aging'. Postural stress testing with spectral analysis of heart rate fluctuations affords a simple, quantitative means of studying cardiovascular dynamics and may provide a useful way of assessing physiologic vs chronologic age.

Acknowledgment. The authors thank D. Rigney for suggestions.

* Supported in part by a grant form NASA/Ames Research Center, Moffett Field, California (A.L. Goldberger).

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0014-4754/87/11/121207-03$1.50 + 0.20/0

Arylsulfatase B synthesis and clearance in inbred mouse strains

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Summary. Arylsulfatase B activity levels were approximately 2–3-fold higher in adult C57BL/6J liver and kidney compared to corresponding tissues from A/J inbred mice. In vivo incorporation of tritiated leucine into C57BL/6J hepatic arylsulfatase B reached a maximum approximately 15 h after injection. The label was cleared from C57BL/6J arylsulfatase B with an apparent half-life of 36 h. The relative rates of synthesis of C57BL/6J and A/J arylsulfatase B were similar; however, the A/J enzyme was cleared more rapidly from liver tissue. C57BL/6J kidney arylsulfatase B appeared to be synthesized at a 2-3-fold higher rate than the corresponding A/J enzyme. These trends suggest genetic regulation of arylsulfatase B is effected through different means in liver and kidney from adult mice of these two inbred strains.
Key words. Arylsulfatase B; lysosomal enzymes; mouse; sulfatase.

Arylsulfatase B (arylsulfate sulfohydrolase EC 3.1.6.1) is a lysosomal hydrolase which catalyzes the hydrolysis of the sulfur to oxygen bond of a variety of synthetic substrates including p-nitrocatechol sulfate (pNCS) and 4-methylumbelliferyl sulfate (4MUS)[1]. The enzyme also hydrolyzes N-acetylgalactosamine 4-sulfate residues from glycosaminoglycans including chondroitin 4-sulfate and dermatan sulfate, and deficiency of arylsulfatase B has been described in patients with Maroteaux-Lamy syndrome and combined sulfatase deficiency disease[2,3].

Relatively little is known regarding regulation of arylsulfatase B expression in human tissues; however, several genes have been described which influence arylsulfatase B activity in murine tissues[4–7]. The structural gene for murine arylsulfatase B *(As-ls)* has been mapped to chromosome 13 near *pe*[8]. A cis-acting regulatory element *(As-lr)* and a trans-acting temporal element *(As-lt)* also map to this region[5,6]. Processing of murine arylsulfatase B, as well as several other acid hydrolases, is affected by *Neu-ls*, the putative structural gene for a liver-specific neuraminidase[7]. Finally, several coat color mutations impair secretion of arylsulfatase B and other lysosomal hydrolases by the kidney proximal tubule[9].

This report describes studies of the in vivo synthesis and clearance of arylsulfatase B from liver and kidney tissues of C57BL/6J (high arylsulfatase B activity) and A/J (low arylsulfatase B activity) inbred mice.

Methods. 5-week-old female C57BL/6J and A/J mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and maintained on commercial feed and water ad libitum for 1–4 weeks prior to use. Mice were grouped in blocks of 4–5 according to strain and injected i.p. with 200 µCi of tritiated L-leucine (140 Ci/mmol, New England Nuclear) and sacri-

Table 1. Relative rate of synthesis of C57BL/6J and A/J arylsulfatase B in liver and kidney

Tissue	Haplotype *As-ls*	*As-lr*	*As-lt*	Activity (units/g)	Protein* (dpm × 10^{-7})	RRI** (×10^5)
Kidney						
C57BL/6J	b	b	b	268 ± 17	5.7 ± 0.2	4.0 ± 0.4
A/J	b	a	a	132 ± 7	6.1 ± 0.3	1.7 ± 0.2
Liver						
C57BL/6J	b	b	b	238 ± 14	7.2 ± 0.3	1.4 ± 0.3
A/J	b	a	a	99 ± 9	6.4 ± 0.4	1.7 ± 0.4

* Estimated from incorporation of tritiated leucine into TCA-precipitable protein from the homogenate (per g wet wt). ** Ratio of incorporation into immunoprecipitable enzyme to incorporation into TCA-precipitable protein. Incorporation into enzyme was corrected for enzyme loss during isolation as described in 'Methods'. Entries represent the mean and SE of 3 experiments.

Table 2. Clearance of arylsulfatase B from C57BL/6J and A/J liver

Time	C57BL/6J RRI / A/J RRI	C57BL/6J activity* / A/J activity
90 min	1.03	2.70
24 h	1.24	3.27
48 h	1.42	2.55
5 days	2.68	2.47

* Expressed as units/g.

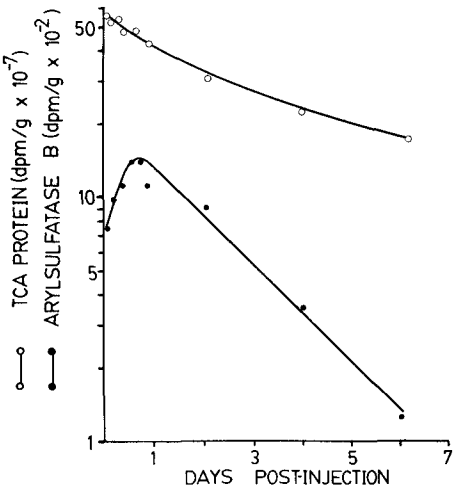
ficed by cervical dislocation. For estimation of the in vivo rate of synthesis, animals were sacrificed 4 h after injection. Appropriate sampling intervals for estimation of clearance of label from hepatic arylsulfatase B were determined with a pilot experiment in which 50 C57BL/6J females were grouped in blocks of five. Each animal was injected i.p. with 200 µCi of tritiated L-leucine, and sacrificed at 1.5, 4, 8, 12, 24, 48, 96, and 144 h after injection. Intervals selected for comparison of in vivo clearance rates were 1.5, 24, 48, and 124 h post-injection. 20 female mice from each inbred strain were grouped and treated as described above to compare their clearance rates. Tissues were excised, rinsed, weighed, and frozen at −20 °C until analysis.

Arylsulfatase B was isolated for incorporation studies using a procedure modified from Berger et al.[10]. 20% w/v homogenates were prepared in 10 mM Tris/HCl/0.15 M NaCl/0.05% v/v Triton X-100 (pH 7.5), and an aliquot was withdrawn for estimation of arylsulfatase B activity and determination of incorporation of label into TCA-precipitable protein. Sodium deoxycholate was added to a final concentration of 0.5% w/v and incubated at 4 °C for 30 min. The pH was lowered to 5.0 by addition of 10% v/v acetic acid, and the mixture was heated at 50 °C for 30 min and cooled. The slurry was centrifuged at 20,000 × g for 30 min, and the supernatant was adjusted to pH 7.4 with 2 M Tris. The supernatant was applied to a 2.5 × 0.5 cm column of Concanavalin A-Sepharose 4B, and arylsulfatase B was eluted with 1 M alpha-methylmannoside. The enzyme was immunoprecipitated using a monospecific, polyclonal anti-arylsulfatase B IgG preparation[11], washed with 0.15 M NaCl, and subjected to Laemmli SDS-electrophoresis[12]. Gels were processed for counting, and incorporation of label into TCA-precipitable protein and arylsulfatase B was determined as described by Smith and Ganschow for murine betaglucuronidase[13]. Correction for enzyme loss during purification was made by dividing the incorporation of label into the immunoprecipitate by the yield of enzyme activity in the Concanavalin A-Sepharose 4B eluate.

Arylsulfatase B activity was assayed using pNCS as substrate[11]. One unit of enzyme activity is defined as µmol pNC released/h.

Results and discussion. Animals were sacrificed four hours after injection to determine the in vivo rate of synthesis of arylsulfatase B (table 1). The relative rate of incorporation (RRI) of arylsulfatase B is expressed as the ratio of incorporation of tritiated leucine into the arylsulfatase B immunoprecipitate to the incorporation of tritiated leucine into TCA-precipitable protein. Recoveries of arylsulfatase B approximated 24% of the homogenate activity and were similar for both strains of mice. Properties of the partially purified enzyme (pH optimum, isoelectric point, response to inhibitors, thermostability, and kinetics) resembled those of the activity present in the crude homogenate. The ratio of the RRI for C57BL/6J kidney arylsulfatase B to that for A/J mice approximated 2.4 which is close to the corresponding ratio of their kidney arylsulfatase B activities. Kidney arylsulfatase B activity differences among inbred strains of mice have been demonstrated to be due to an allelic difference at the *As-lr* site which influences the concentration of the enzyme determined by the structural locus on the same chromosome[5]. The present data suggest that the *As-lr* element influences the rate of synthesis of arylsulfatase B. By contrast, the RRI for hepatic arylsulfatase B from C57BL/6J and A/J mice were comparable, whereas the ratio of their hepatic arylsulfatase B activities approximated 2.4 (table 1). These trends suggest that regulation of liver arylsulfatase B expression is more complicated than that for the kidney isozyme.

Incorporation of label into and clearance from C57BL/6J hepatic arylsulfatase B was measured to determine appropriate sampling intervals for comparison of C57BL/6J and A/J arylsulfatase B clearance rates (fig.). Label was gradually cleared from the total protein pool with an apparent half-life of 3.5 days. Approximately 15 h were required for optimal labeling of arylsulfatase B to occur. The label was subsequently cleared from the enzyme with an apparent half-life of 36 h. This would necessarily constitute on overestimate, however, since some recycling of label would be anticipated to occur. No evidence for lower molecular weight forms that may have been produced by degradation of the enzyme was observed.



Incorporation of tritiated leucine into total protein (TCA protein) and arylsulfatase B from C57BL/6J liver. 50 female C57BL/6J mice were injected i.p. with 200 µCi of tritiated leucine per mouse and treated as described in 'Methods'. Incorporation of label into arylsulfatase B has been corrected for loss during purification as described in 'Methods'.

Clearance of the C57BL/6J and A/J hepatic enzymes is presented in table 2. The data are entered as ratios of the C57BL/6J RRI to the A/J RRI at 90 min, 24 h, 48 h, and 5 days post injection. Since label was cleared from total protein at comparable rates by the two inbred strains (data not shown), a progressive increase in these ratios over the interval 90 min to 5 days would support more rapid clearance of the A/J enzyme. This trend is apparent in table 2. These data suggest that the 2–3-fold higher arylsulfatase B activity present in C57BL/6J liver is largely due to a longer half-life of the enzyme in liver from this inbred strain compared to that in A/J liver.

Cis- and trans-acting genetic elements have been reported which influence activities of a number of other murine lysosomal hydrolases. The beta-galactosidase structural gene *(Bgl-e)* is subject to both a cis-acting regulatory element *(Bgl-s)* and a trans-acting temporal element *(Bgl-t)*. Both of these elements are closely linked to or within *Bgl-e* and appear to affect the rate of synthesis of beta-galactosidase[10]. Beta-galactosidase activity is also influenced by a trans-acting temporal element unlinked to *Bgl-e*[14]. This gene also affects the rate of enzyme synthesis. Regulation of beta-glucuronidase activity has also been extensively studied, and a cis-acting element, *Gus-r*, affects androgen induction of enzyme activity in kidney proximal tubule cells by influencing the levels of translatable glucuronidase mRNA[15]. Other sites within the glucuronidase gene complex have been identified which appear to increase the rate of clearance of glucuronidase from tissues of certain inbred murine strains[16]. The present report indicates that both rates of synthesis and clearance of murine arylsulfatase B differ among inbred strains. Furthermore, although C57BL/6J mice possess more than 2-fold greater arylsulfatase B activity in both liver and

kidney, the predominant mechanisms responsible for maintaining the interstrain activity variation are different in the respective tissues.

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0014-4754/87/11/121209-03$1.50 + 0.20/0

Synthesis of three NH_2-terminally extended arginine-vasopressins with prolonged biological activities

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Summary. The synthesis of three novel AVP-analogues, extended by 1–3 amino acids at their NH_2-termini in accordance with the sequence of the bovine arginine-vasopressin neurophysin II precursor, is reported. The compounds were assayed for their antidiuretic and vasopressor activities with particular attention to the duration of the effects. All compounds showed high potency, based on the intensity, and prolonged effects in both test systems compared with AVP.
Key words. Prolonged vasopressin effect; solid phase peptide synthesis; vasopressin analogue; vasopressin precursor.

Protraction of peptide hormone activity with the aid of synthetic analogues has been attempted and accomplished according to various principles. In the vasopressin field one successful approach involved the preparation of hormonogens[1,2], from which, it was claimed, the hormone was continuously generated by gradual enzymatic degradation[3,4]. Thus, among several NH_2-terminally extended lysine-vasopressins with prolonged effects, Gly-Gly-Gly-lysine-vasopressin has found clinial applications in the treatment of bleeding disorders[5] and is marketed under the name of Glypressin.

With the recent publication of the sequence of the bovine arginine-vasopressin neurophysin II precursor[6], it became feasible to make and examine related vasopressin analogues with primary structures derived from this precursor. This paper describes the synthesis and some biological properties of three such analogues, Ala-AVP (I), Ser-Ala-AVP (II) and Thr-Ser-Ala-AVP (III). In this context it should be pointed out that the human vasopressin-containing gene has now also been sequenced[7]. Human AVP is immediately preceded by Ser-Ser-Ala instead of Thr-Ser-Ala.

Material and methods. The three protected peptides corresponding to the final products I–III were synthesized by the solid phase method[8,9] in the manual mode. When not otherwise stated, the general experimental conditions were as described previously[10]. Starting from 2.42 g of Boc-Gly-resin with a load of 0.62 mmol Gly/g[10], after 8 cycles, including deprotections, neutralizations and couplings with intermittent washings, 4.40 g of Boc-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-resin was obtained. All coupling steps were monitored by the Kaiser test.[11]. At this stage the resin was divided into 3 equal parts. The first one after another cycle with Z-Ala as carboxyl component, ammonolysis[9], extraction with warm DMF, precipitation with water followed by reprecipitation from DMF-EtOH-ether, gave Z-Ala-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (Ia) as a white powder. For further synthetic details and physical data, see table 1. Z-Ser(Bzl)-Ala-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg-(Tos)-Gly-NH_2 (IIa) and Thr(Bzl)-Ser(Bzl)-Ala-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 (IIIa) were prepared similarly from the second part through 2 cycles with Boc-Ala and Z-Ser(Bzl) and from the third part through 3 cycles with Boc-Ala, Boc-Ser(Bzl) and Boc-Thr(Bzl) as carboxyl components, respectively, followed in the last case by deprotection with HCl/HOAc, with further treatment of the resins as described for compound Ia. Data on IIa and IIIa are also given in table 1.

Aliquots of Ia, IIa and IIIa (200 mg), respectively, were treated in 400 ml of redistilled, sodium-dried ammonia at its boiling point under stirring with sodium, contained in a small-bore glass tube, until the blue color persisted for 30 s. Glacial acetic acid (0.40 ml) was added, the ammonia evaporated in vacuo, the residue dissolved in 0.2% aq. HOAc (800 ml) and titrated with 2 M NH_3 to pH 6.5. Excess $K_3Fe(CN)_6$ (0.01 M, 23 ml) was added gradually under stirring to give a yellow solution which was stirred for 10 min with an anion-exchange resin (Amberlite IR-45, acetate cycle, 20 g damp). After filtering and washing with 0.2% HOAc (100 ml), the combined extract was lyophilized to give a powder which was desalted on a Sephadex G-15 column (115 × 2.9 cm) in 50% HOAc (flow-rate 5.5 ml/h, monitoring at 254 nm). Pertinent fractions were pooled, lyophilized and again chromatographed on Sephadex G-15 (130 × 1.4 cm) in 0.2 M HOAc (flow-rate 3 ml/h) and after pooling and lyophilizing finally chromatographed on Sephadex LH-20 (135 × 1.8 cm) in 0.2 M HOAc to give pure I, II and III, respectively; these are further characterized in table 1.

Antidiuretic assays were performed in anesthetized hydrated