Acta Biochimica Polonica

Vol. 49 No. 4/2002

927–936

QUARTERLY

# Species- and substrate-specific stimulation of human plasma paraoxonase 1 (PON1) activity by high chloride concentration✪

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Received: 28 May, 2002; revised: 28 October, 2002; accepted: 21 November, 2002

**Key words:** paraoxonase, arylesterase, lipid peroxidation, oxidative stress

**Paraoxonase 1 (PON1), contained in plasma high-density lipoproteins, plays an important role in the protection of plasma lipoproteins and cell membranes from oxidative damage. Previous studies indicate that human PON1 is stimulated by high NaCl concentrations. The aim of this study was to characterize in more detail the effect of salts on serum PON1. Paraoxon-hydrolyzing activity of human serum was stimulated by 81.6% following the addition of 1 M NaCl. The effect of NaCl was dose-dependent between 0.5 and 2 M. PON1 activity toward phenyl acetate was reduced by 1 M NaCl by 55.2%. Both the paraoxon- and phenyl acetate-hydrolysing activity was slightly lower in heparinized plasma than in serum, but NaCl had similar stimulatory and inhibitory effects on these activities, respectively. In rat, rabbit, and mouse, NaCl reduced PON1 activity. KCl had a similar effect on human PON1 as NaCl. Sodium nitrite also stimulated human PON1 but much less effectively than chloride salts. In contrast, sucrose, sodium acetate and sodium lactate had no significant effect. NaBr was a less effective PON1 activator than NaCl, whereas the effect of NaJ was non-significant. The activity of human PON1 toward homogentisic acid lactone and $\gamma$-decanolactone was unaltered by NaCl. These data indicate that: 1) high concentrations of chlorides stimulate human PON1 activity toward paraoxon but not other substrates, 2) PON1 is inhibited by $Cl^-$ in other mammalian species, 3) the potency of human PON1 activation by halogene salts increases with decreasing atomic mass of the halide anion.**

Paraoxonase 1 (PON1) is a calcium-dependent "A-esterase" contained in plasma high-density lipoproteins (HDL), which hydrolyzes toxic acetylcholinesterase-inhibiting organic phosphates, such as paraoxon (La Du *et al.*, 1993; Mackness *et al.,* 1996). Studies performed during the last 10 years indicate that PON1 has multiple other important functions. PON1 protects low-density lipoproteins from oxidative modification by reactive oxygen spe-

✪This study was supported by grant PW 447/2002 from the Medical University, Lublin, Poland.

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**Abbreviations:** AE, arylesterase; HDL, high-density lipoproteins; PON1, paraoxonase 1.

cies and thus significantly contributes to the atheroprotective effect of HDL (Mackness *et al.,* 1991; Mackness *et al.,* 1993; Watson *et al.,* 1995). The enzyme also hydrolyzes phospholipid hydroperoxides and cholesterol ester hydroperoxides (esterase activity) and reduces lipid hydroperoxides to the respective hydroxides as well as degrades hydrogen peroxide (peroxidase activity) (Aviram *et al.,* 2000). PON1 also protects HDL from peroxidation and improves reverse cholesterol transport to the liver (Aviram *et al.,* 1998b). It is also suggested that PON1 protects plasma membranes from free radical injury (Durrington *et al.,* 2001). The enzyme degrades bioactive phospholipids, such as platelet-activating factor (PAF) (Rodrigo *et al.,* 2001). Recent studies indicate that PON1 possesses lactonase activity and is involved in the metabolism of some drugs such as statins, spironolactone and glucocorticoid lactones (Billecke *et al.,* 2000). Finally, it hydrolyzes homocysteine thiolactone and prevents protein homocysteinylation, a process involved in atherogenesis (Jakubowski, 2000). Two closely related proteins: PON2 and PON3 have been identified. PON3 is also contained in HDL particles (Draganov *et al.,* 2000; Reddy *et al.,* 2001) whereas PON2 is absent in plasma but is expressed in many tissues (Ng *et al.,* 2001). Both PON2 and PON3 possess antioxidant properties and lactonase activity, but unlike PON1, they lack the paraoxon- or phenyl acetate-hydrolyzing activity.

Early studies indicated that the paraoxon- but not phenyl acetate-hydrolyzing activity of human plasma PON1 is activated by high concentration of NaCl (Eckerson *et al.,* 1983a; 1983b). However, neither the effect of other salts nor the mechanism of this stimulation have been investigated. Recent data indicate that basal and NaCl-stimulated PON1 activity may be differentially regulated under pathologic conditions, which suggests a possible physiologic and diagnostic significance of PON1 measurement in the presence of NaCl (Schiavon *et al.,* 1996).

The purpose of this study was to examine more extensively PON1 stimulation by NaCl. We investigated this effect in different mammalian species, compared the effect of different salts and evaluated the effect of NaCl on other PON1 activities, such as arylesterase and lactonase activity.

## MATERIALS AND METHODS

***Sample collection.*** Blood was obtained from 15 male healthy volunteers at the age of 28–54 years without any chronic diseases and taking no medications. Neither of them suffered from acute infection during 4 weeks before the study and neither had a history of atherosclerosis risk factors known to affect PON1 activity (diabetes mellitus, hyperlipidemia, smoking, arterial hypertension). Blood was withdrawn by venipuncture after overnight fasting. All persons were normolipidemic (plasma cholesterol and triglycerides < 200 mg/dl), normoglycemic (glucose < 100 mg/dl) and normotensive (RR < 140/90 mmHg) at the time of the study.

Animal studies were performed on adult male Wistar rats, white New Zealand rabbits and Swiss mice (n = 10 for each species). Blood was obtained from the rats from the abdominal aorta under pentobarbital anaesthesia (50 mg/kg i.p.). In rabbits, blood samples were withdrawn from the ear vein. From mice blood was collected after decapitation.

Blood samples were collected to glass tubes, allowed to clot for 1 h at room temperature and was centrifuged for 10 min at 3000 × ***g*** at 4°C. Serum was separated, frozen and stored at –25°C until assay. Separate samples were withdrawn into heparinized tubes (for PON1 assay in plasma) and into EDTA-containing tubes (for plasma lipid profile and glucose).

The study protocol was reviewed and approved by the local ethics committee.

***Measurement of PON1 activity.*** PON1 activity toward paraoxon (diethyl-*p*-nitrophenyl phosphate) was determined by mea-

suring the initial rate of substrate hydrolysis to *p*-nitrophenol, whose absorbance was monitored at 412 nm in the assay mixture (800 $\mu$l) containing 2.0 mM paraoxon, 2.0 mM $CaCl_2$ and 20 $\mu$l of serum or plasma in 100 mM Tris/HCl buffer (pH 8.0). A blank sample containing incubation mixture without plasma was run simultaneously to correct for spontaneous substrate breakdown. The enzyme activity was calculated from $\varepsilon_{412}$ of *p*-nitrophenol (18290 $M^{-1} \cdot cm^{-1}$) and was expressed in U/ml; 1 U of enzyme hydrolyzes 1 nmol of paraoxon/ min (Schiavon *et al.,* 1996; Ayub *et al.,* 1999).

The activity toward phenyl acetate (arylesterase activity) was determined by measuring the initial rate of substrate hydrolysis in the assay mixture (3 ml) containing 2 mM substrate, 2 mM $CaCl_2$ and 10 $\mu$l of serum or plasma in 100 mM Tris/HCl (pH 8.0). The absorbance was monitored for 3 min at 270 nm. Blank sample prepared as described above but without plasma, representing nonenzymatic hydrolysis, was subtracted and the activity was calculated from $\varepsilon_{270}$ = 1310 $M^{-1} \cdot cm^{-1}$. The results are expressed in U/ml, 1 U of arylesterase hydrolyzes 1 $\mu$mol of phenyl acetate per minute (Schiavon *et al.,* 1996, Ayub *et al.,* 1999).

PON1 is also able to hydrolyze aromatic and aliphatic lactones. We assayed the activity toward homogentisic acid lactone and $\gamma$-decanolactone representing these two groups, respectively, because these compounds are vigorously metabolized by PON1, and other PONs have little contribution to their hydrolysis by serum (Reddy *et al.,* 2001). The activity toward homogentisic acid lactone was assayed in 50 mM Tris/HCl (pH 8.0) containing 1 mM $CaCl_2$ and 1 mM substrate (from 100 mM stock solution in methanol). The absorbance at 290 nm was monitored and the rate of hydrolysis was calculated from the difference between the absorption coefficients of the acid formed and the lactone (816 $M^{-1} \cdot cm^{-1}$) (Draganov *et al.,* 2000; Billecke *et al.,* 2000).

The hydrolysis of $\gamma$-decanolactone was measured in 2 mM Hepes buffer (pH 8.0) containing 1 mM $CaCl_2$, 0.004% phenol red, 0.005% bovine serum albumin and 1 mM substrate (from 100 mM stock solution in methanol). The increase in absorbance was monitored at 422 nm after addition of sample and the rate of hydrolysis was calculated from the calibration curve obtained using known amounts of HCl. The activity was expressed in moles of acid produced by 1 ml of serum in 1 min (Billecke *et al.,* 2000).

All measurements were performed at 25°C. The effect of salts on PON1 activity was assayed in the respective buffers containing additionally the investigated salt at the appropriate concentration. These buffers were prepared separately and their pH was always adjusted to 8.0.

Paraoxon, phenyl acetate, homogentisic acid lactone, $\gamma$-decanolactone, phenol red, bovine serum albumin, Tris and HEPES were obtained from Sigma-Aldrich. All other reagents were of research grade. Plasma cholesterol, triglycerides and glucose were assayed using Sigma-Aldrich kits.

Because due to genetic polymorphism human serum paraoxonase activity demonstrates trimodal distribution, statistical analyses were performed by nonparametric Wilcoxon's test. The relationship between variables was analyzed by Spearman rank correlation test. $P < 0.05$ was considered significant. Data in the text are reported as median (range).

The degree of homology of PON1 in different mammalian species was calculated on the basis of amino-acid sequences of the human, rat, rabbit and mouse enzymes obtained from the SWISS-PROT protein sequence database on the Expert Protein Analysis System (ExPASy) Molecular Biology Server (www.expasy.org).

## RESULTS

### The effect of NaCl on human PON1

The paraoxon-hydrolyzing activity of human serum differed markedly between individuals, consistently with other studies indicating that a common genetic PON1 polymorphism (arginine or glutamine at position 192) affects the enzyme activity toward paraoxon (Eckerson *et al.,* 1983a; Aviram *et al.,* 1998a). Within the study group, the activity toward paraoxon ranged from 25.2 to 236.7 U/ml (median: 68.5 U/ml). In all individuals 1 M NaCl caused marked stimulation of the paraoxon hydrolyzing activity. The PON1 activity in the presence of NaCl was 49.9–462.6 U/ml (median: 127.4 U/ml), i.e. 41.0–169.9% (median: 81.6%) higher than in the absence of this salt (Fig. 1). The PON1 activity toward paraoxon measured in heparinized plasma was by 3.7–19.4% (median 12.2%) lower than in serum (Fig. 1). After addition of 1 M NaCl, the plasma PON1 activity increased to 23.7–236.6 U/ml (median 52.7 U/ml), i.e. by 45.2–140.6% (median 92.8%). However, the NaCl-stimulated activity in the plasma was 6.3–14.3% (median 8.6%) lower than the NaCl-stimulated activity in the serum (Fig. 1). A very strong correlation between the PON1 activity in serum and plasma was observed both in the absence (r = 0.995, $P < 0.001$) and in the presence (r = 0.996, $P < 0.001$) of NaCl. In contrast to heparinized plasma and consistently with the $Ca^{2+}$-dependence of PON1, very low activity was detected in plasma obtained using EDTA as an anticoagulant.

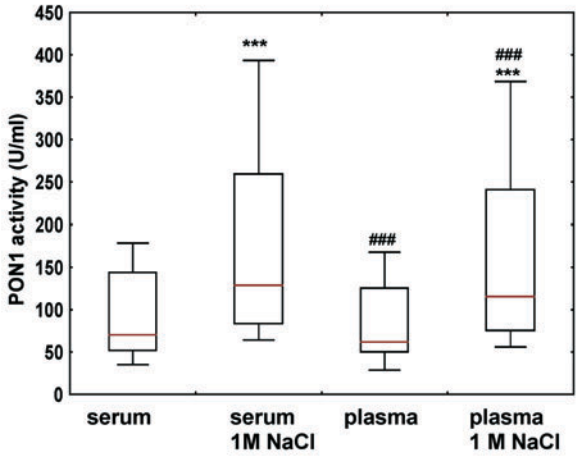


**Figure 1. Box and whiskers plot of human PON1 activity toward paraoxon in serum and plasma in the absence and in the presence of 1 M NaCl.**

Boxes indicate median, 25 and 75 percentiles and whiskers represent 10 and 90 percentile. $^{***}P < 0.001$ (compared to the activity measured without NaCl); $^{###}P < 0.001$ (compared to the activity in serum) by nonparametric Wilcoxon test.

The absolute (expressed in U/ml) increase in PON1 activity after addition of salt correlated significantly with the basal unstimulated activity (r = 0.84, $P < 0.001$).

The effect of NaCl on the paraoxon-hydrolysing activity was dose-dependent (Fig. 2).

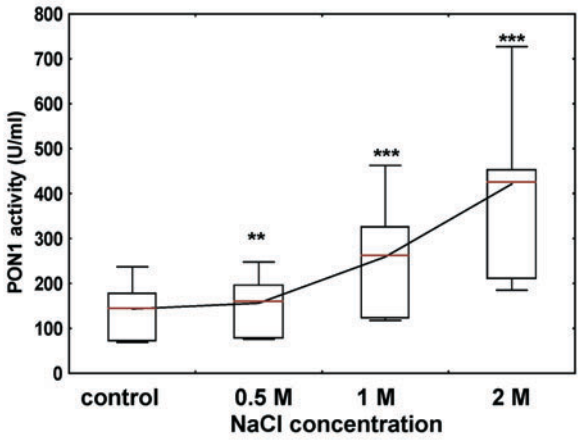


**Figure 2. Dose-dependent effect of NaCl on human serum PON1 activity toward paraoxon.**

$^{**}P < 0.01$, $^{***}P < 0.001$ (compared to control by nonparametric Wilcoxon's test).

NaCl at a concentration of 0.5 M stimulated the enzyme by 4.6–11.0% (median 9.6%), whereas 2 M concentration increased its activity by 154.0–207.0% (median 194.2%).

The PON1 activity toward phenyl acetate (arylesterase activity, AE) was much less variable in the study group (median: 171.9 U/ml, range: 127–207.2 U/ml) consistently with the fact that this activity is not affected by the genetic polymorphism. Addition of 1 M NaCl to

the assay mixture markedly reduced the AE activity 26.0–114.4 U/ml (median 77.6 U/ml), i.e. to 20.5–57.9% (median 55.2%) (Fig. 3). As in the case of paraoxon, the phenyl acetate-hydrolyzing activity of heparinized plasma was by 3.8–19.4% (median 12.8%) lower in comparison to serum. NaCl reduced

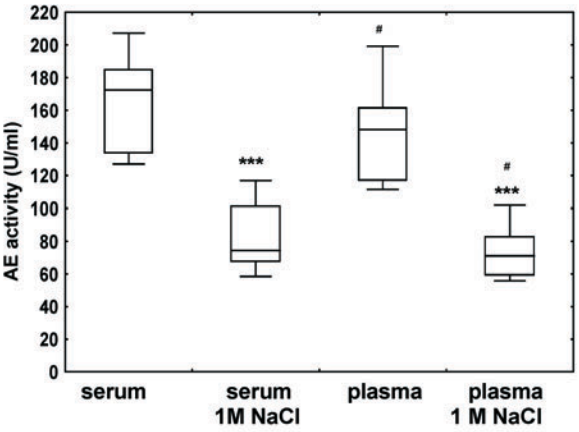


**Figure 3. Human PON1 activity toward phenyl acetate (arylesterase activity, AE) in serum and plasma in the absence and in the presence of 1 M NaCl.**

***$P < 0.001$ (compared to the activity measured without NaCl); #$P < 0.05$ (compared to the activity in serum) by nonparametric Wilcoxon's test.

the AE activity in the plasma by 25.9–62.5% (median 51.9%). The AE activity in the plasma correlated significantly with the AE activity in serum both in the absence (r = 0.89, $P = 0.007$) and in the presence (r = 0.83, $P = 0.009$) of NaCl. Very low AE activity was noticed in EDTA plasma.

### The effect of NaCl on PON1 in animals

The above results indicate that high concentration of NaCl stimulates only the paraoxonbut not the phenyl acetate-hydrolyzing activity of human PON1. To test whether this effect appears also in other mammalian species, we investigated the effect of NaCl on the rat, rabbit, and murine enzyme (Fig. 4). In contrast to human serum, 1 M NaCl reduced the PON1 activity in rat serum by 7.9–26.9% (median 16.1%). The enzyme activity in rat heparinized plasma was 3.8–19.5% (median 12.8%) lower than in the serum and was further inhibited following the addition of NaCl to the assay mixture by 6.3–14.3% (median 7.6%). The AE activity in rat serum was lowered by NaCl from 142.6 (108.3–156.1 U/ml) to 51.7 (42.1–63.4 U/ml), i.e. by 55.5–66.8% (median 62.3%, $P < 0.001$). Rat heparinized plasma demonstrated lower AE activity than serum (median: 134.1 U/ml, range: 92.8–141.7 U/ml, $P < 0.05$) and this was further decreased following the addition of 1 M NaCl to 43.8 (32.1–57.9 U/ml, $P < 0.001$ *vs* the activity in the plasma without NaCl, $P < 0.05$ *vs* the activity in the serum with NaCl).

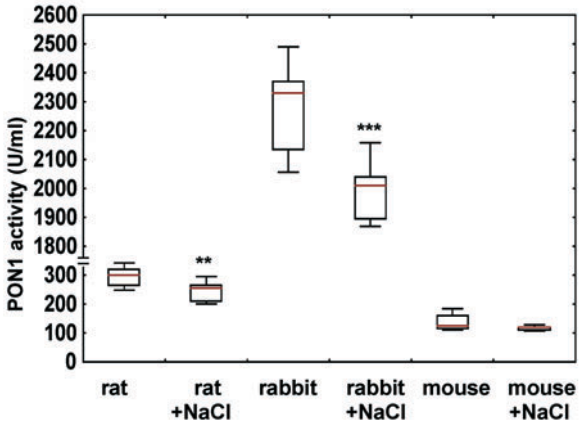


**Figure 4. The effect of 1 M NaCl on paraoxon-hydrolyzing activity of serum from different mammalian species.**

**$P < 0.01$, ***$P < 0.001$ (compared to the activity measured without NaCl by nonparametric Wilcoxon's test).

Interestingly, a very high PON1 activity toward paraoxon was noticed in the rabbit serum (Fig. 4). The activity assayed in the presence of NaCl was 16.1–54.4% (median 22.3%) lower than in the absence of this salt. In the mouse serum, NaCl decreased the enzyme activity in 9 of the 10 animals studied (maximally by 37.4%), in one animal a slight 2.4% stimulation of the activity was observed. When all ten samples were taken into account, the median NaCl-induced change in the paraoxon-hydrolyzing activity was –7.6% and

was close to, but did not reach the level of significance ($P = 0.06$). Thus in all the animal species studied, NaCl reduced or tended to reduce PON1 activity, although the sensitivity of the enzyme to this inhibition slightly differed among species. The stimulation by high NaCl concentration is a unique feature of human paraoxonase.

The arylesterase activity was lowered by 1 M NaCl in the rabbit serum from 912.5 (873.0–935.6 U/ml) to 403.5 (298–309 U/ml), i.e. by 66.7% (range: 65.7–67.2%, $P < 0.001$) and in the mouse serum from 59.3 (52.6–63.9 U/ml) to 25.8 (25.1–26.6 U/ml), i.e. by 57.5% (range: 50.0–58.4%, $P < 0.01$). It should be noted that the arylesterase activity was markedly more sensitive to inhibition by NaCl than the paraoxon-hydrolyzing activity in all mammalian species studied.

### The effect of other salts and nonelectrolytes on PON1

To investigate whether the stimulation of human PON1 by NaCl is a specific effect of given ions or a nonspecific phenomenon resulting from high osmolality and/or ionic strength of the solution, we studied the effect of other compounds on PON1 activity toward paraoxon (Fig. 5). In this experiment, 1 M NaCl increased the enzyme activity by 79.9% (range: 41.0–121.1%). Potassium chloride caused a comparable increase in PON1 activity (median: 96.7%, range 42.5–315.2%). In contrast, sodium acetate had no significant effect on the paraoxon-hydrolyzing enzyme. Also sodium lactate did not change the PON1 activity. $NH_4Cl$ was a less effective activator of PON1 than either NaCl or KCl. The activity measured in the presence of this salt increased by 54.0% (range: 2.4–98.5%). Sodium nitrite also increased the PON1 activity but only by 32.6% (0.3–54.3%). The effect of sodium nitrate was comparable to sodium nitrite. Sucrose (2 M) did not change the PON1 activity. Addition of 1 M $MgCl_2$ or 1 M $NaH_2PO_4$ caused almost complete inhibition of PON1 activity, most likely due to the displacement of calcium from PON1 molecules by $Mg^{2+}$ and $Ca^{2+}$ binding in the solution by $H_2PO_4^-$, respectively.

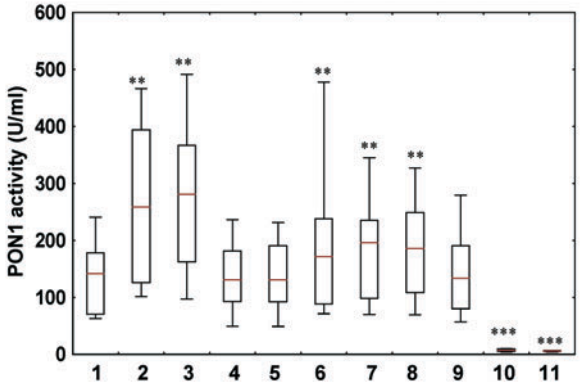


**Figure 5. The effect of different compounds on human serum PON1 activity toward paraoxon.**

1, control; 2, NaCl; 3, KCl; 4, sodium acetate; 5, sodium lactate; 6, $NH_4Cl$; 7, $NaNO_2$; 8, $NaNO_3$; 9, sucrose; 10, $MgCl_2$; 11, $NaH_2PO_4$. Sucrose was added at 2 M, other compounds at 1 M. $^{**}P < 0.01$, $^{***}P < 0.001$ (compared to control by nonparametric Wilcoxon's test).

Human serum AE activity was decreased by 1 M KCl from 171.9 (134.1–207.7 U/ml) to 125.0 (90.2–150.7 U/ml), i.e. by 26.5% (18.6–32.8%, $P < 0.01$). Thus, KCl and NaCl

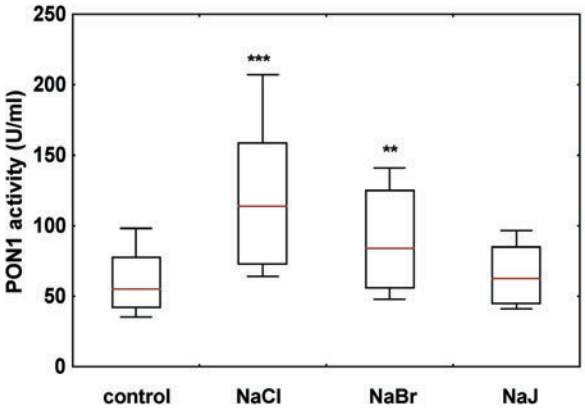


**Figure 6. The effect of sodium halides on human serum PON1 activity toward paraoxon.**

$^{**}P < 0.01$, $^{***}P < 0.001$ (compared to control by nonparametric Wilcoxon's test).

caused a comparable degree of inhibition. Sodium acetate (1 M) decreased the AE activity much more markedly, to 80.7 (64.1–98.3 U/ml), i.e. by 52.2% (51.2–53.5%, $P < 0.001$). In contrast, 2 M sucrose inhibited human serum AE to 151.0 (115.7–188.3 U/ml), i.e. only by 9.4% (8.4–14.2%, $P < 0.05$).

The paraoxon-hydrolyzing activity of the rat and rabbit sera was inhibited by 1 M KCl to a similar degree as by NaCl. In contrast, sodium acetate decreased the rat and rabbit PON1 activity by 30–40%. Sucrose (2 M) tended to stimulate PON1 slightly in both these species, but the effect was not significant.

### The effect of different halides on human PON1

The potent stimulation of human serum PON1 by $Cl^-$-containing salts prompted us to compare the effect of different halides (Fig. 6). As can be seen in this figure, NaBr was a less potent activator of PON1 than NaCl whereas the effect of NaJ was non-significant. These data suggest that the stimulation of human PON1 by sodium halides increases with the decreasing atomic mass of the halogene ion. Surprisingly, vigorous spontaneous hydrolysis of paraoxon without addition of serum was observed in the solution containing 1 M NaF. Therefore, it was not possible to measure the enzyme activity in the presence of this salt.

### The effect of NaCl on lactonase activity of human PON1

Finally, we investigated whether NaCl affects also the lactonase activity of human PON1. For this purpose, we measured the homogentisic acid lactone- and $\gamma$-decanolactone-hydrolysing activity of human serum. We observed no significant difference between these activities assayed in the absence and in the presence of 1 M NaCl (homogentisic acid-hydrolyzing activity without NaCl: median 9.11 $\mu$mol/min per ml, range 5.31–18.79 $\mu$mol/min per ml; homogentisic acid-hydrolyzing activity with 1 M NaCl: median 11.00 $\mu$mol/min per ml, range 7.20–16.34 $\mu$mol/min per ml; $\gamma$-decanolactone hydrolyzing activity without NaCl: median 7.72 $\mu$mol/min per ml, range 4.86–10.20 $\mu$mol/min per ml; $\gamma$-decanolactone-hydrolyzing activity in the presence of 1 M NaCl: median 7.70 $\mu$mol/min per ml, range 4.56–9.72 $\mu$mol/min per ml). Thus, the effect of NaCl on human PON1 depends on the hydrolyzed substrate and ranges from inhibition (phenyl acetate) through no change (lactones) to stimulation (paraoxon).

## DISCUSSION

The results of this study confirm that the activity of human PON1 toward paraoxon is stimulated by high NaCl concentration (Eckerson *et al.,* 1983a; 1983b). In contrast, NaCl reduced the PON1 activity toward phenyl acetate and did not change its lactonase activity. Previous studies (Eckerson *et al.,* 1983a; 1983b) demonstrated that only the R phenotype (arginine at position 192) was activated by NaCl, whereas the Q phenotype (glutamine at this position) was not. In the present experiment we observed stimulation of PON1 in all studied individuals. We did not determine the PON1 genotype in our study. The R and Q phenotypes can also be discriminated on the basis of the ratio between the enzyme activity toward paraoxon and toward phenyl acetate. However, this requires a larger population to be done reliably (Geldmacher von Mallinckrodt *et al.,* 1983; Juretic *et al.,* 2001). Because we observed a broad range of PON1 activities, we suppose that both phenotypes were represented in the study group. A strong correlation was noted between the baseline activity and the NaCl-stimulated increase in activity, which suggests that the R phenotype (characterized by higher paraoxon-hydrolyzing activity) was stimulated by NaCl more than the Q phenotype.

PON1 activity is examined in either serum or heparinized plasma (Hasselwander *et al.,* 1999). We have demonstrated that the PON1 activity is slightly lower in heparinized plasma than in serum. However, human PON1 is stimulated by NaCl in both serum and plasma, and a very strong correlation exists between the PON1 activities in both fluids in the absence as well as in the presence of NaCl. Thus, we suggest that either serum or heparinized plasma may be used for the enzyme assay.

In contrast to humans, PON1 was suppressed by NaCl in other mammalian species studied. It is unclear at present what determines this specific effect of NaCl on the human enzyme. The amino-acid sequence of rat, rabbit and mouse PON1 demonstrates 79.9%, 85.3% and 82.3% homology with human PON1, respectively. Even small structural differences can affect the paraoxon-hydrolyzing activity and the effect of NaCl, as evidenced by the higher sensitivity to the NaCl-induced stimulation of human R in comparison with the Q isoenzyme differing in only one residue (Eckerson *et al.,* 1983a). Apart from the structure of paraoxonase itself, species-specific composition of other HDL components may be responsible for this unique feature of the human enzyme, because binding to phospholipids and apolipoprotein AI is essential to maintain PON1 activity (Sorenson *et al.,* 1999).

The results of studies comparing the effect of different salts and sucrose on human PON1 (Fig. 5) indicate that: 1) neither salts of organic anions nor nonelectrolytes had any significant effect on human PON1, thus the stimulation by NaCl does not result from either osmolality or ionic strength of the solution; 2) the effect of NaCl is not specific for this salt, however, among inorganic salts chlorides seem to be the most effective activators, although the accompanying cation also has some significance (weaker effect of $NH_4Cl$ *vs* NaCl and KCl).

The AE activity was equipotently inhibited by NaCl and KCl. Sodium acetate caused more marked inhibition whereas sucrose only slightly decreased human AE activity. These results indicate that high ionic strength of the solution, not high osmolality, is responsible for AE inhibition by high salt concentration. The difference between the effects of NaCl or KCl and sodium acetate suggests the possibility that $Cl^-$ has also the ability to stimulate the enzyme activity toward phenyl acetate, however, this is abolished by the nonspecific inhibitory effect exerted for by high ionic strength of the solution. In contrast, the activity toward paraoxon is less sensitive to the effect of ionic strength because it is unaltered or even slightly increased by other salts, which results in marked stimulation of this activity by $Cl^-$. This hypothesis, however, requires confirmation by further studies.

The physiologic significance of PON1 activation by NaCl is unclear. The salt concentration required to cause significant changes in the enzyme activity is far above that observed *in vivo*. In addition, the paraoxon-hydrolysing activity does not necessarily correlate with the antioxidant properties of paraoxonase. For example, genetic polymorphism of human PON1 has opposite effects on paraoxon hydrolysis and the antioxidant properties. The R isoenzyme has a higher paraoxon-hydrolysing activity than the Q isoenzyme, but is less effective in preventing lipoprotein oxidation (Mackness *et al.,* 1997; 1998). The antioxidative PON1 activity is dependent on a different active site than its esterase activity. Inhibition of LDL oxidation by PON1 requires free SH groups, and is $Ca^{2+}$-independent, whereas the esterase activity requires $Ca^{2+}$ but not free SH groups (Aviram *et al.,* 1998a). To our knowledge, the effect of NaCl on the antioxidant activity of PON1 has not been studied so far. This would, however, require purification of paraoxonase because other components of HDL, such as platelet-activating factor acetylhydrolase (PAF-AH) and lecithin : cholesterol acyltransferase (LCAT) also have antioxidant properties (Mackness & Durrington, 1995). Isolation of PON1 from its natural

microenvironment could, on the other hand, modify the effect of salts. The lactonase activity which is suggested to better correlate with the physiologic enzyme function (Billecke *et al.,* 2000) did not change in the presence of the NaCl in this study. Thus, the physiologic significance of the NaCl-induced stimulation is unclear.

However, some studies suggest that the basal and NaCl-stimulated PON1 activities are differentially affected in pathologic conditions. For example, Schiavon *et al.* (1996) found that the basal but not the NaCl-activated enzyme activity is reduced in hemodialyzed patients. We have recently found (unpublished observation) that the paraoxon-hydrolyzing activity of human serum decreases following surgical trauma if measured in the absence but not in the presence of NaCl. These data suggest that under some conditions, the basal PON1 activity is more sensitive to modifying factors than the NaCl-stimulated activity. It would be interesting to recognize factors which determine the enzyme activity in the absence as well as in the presence of NaCl. It is likely that the changes of activity observed under both conditions indicate more profound abnormalities than changes of only the basal but not the stimulated activity. Further studies addressing the PON1 activity under different physiologic and pathologic conditions in the absence as well as in the presence of NaCl are needed to verify this possibility.

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