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SHORT COMMUNICATIONS

Effects of phenylarsine oxide on agonist-induced hepatic vasoconstriction and glycogenolysis*

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Arsenic is a common element with a distinguished and ancient history of toxicity. The molecular basis of its toxicity is related to its oxidation state, dosage, and chemical form [1]. Several studies have shown that, following arsenical exposure, the liver and kidney contain high concentrations of arsenic [2–4]. Arsenical intoxication has been shown to perturb hepatic function in both human and animal studies [5–9]. Chronic exposure to arsenicals was correlated with portal hypertension [10–12], and Datta *et al.* speculated that arsenicals may interact specifically with the portal vein subendothelium to elicit portal fibrosis [12].

In addition to being a major site for xenobiotic detoxification, the liver is involved in regulating blood glucose levels through glycogenolysis and gluconeogenesis in the fed and fasted state respectively. Regulation *in vivo* of these latter enzymatic pathways is achieved by hormonal and non-hormonal mechanisms. Glycogenolysis stimulated by hormones (i.e. glucagon, adrenergic agonists, vasopressin) occurs through activation of cAMP- or Ca^{2+}-dependent protein kinases, whereas non-hormonal activation of glycogenolysis, particularly during hypoxic or ischemic situations, appears to be effected through elevated cytosolic AMP concentrations which activate both phosphorylase *b* and phosphorylase kinase [13].

Recently, several autacoid mediators, including platelet activating factor (AGEPC, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), prostaglandin E_2, and a thromboxane A_2-mimetic (U-46619, 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$) have been shown to stimulate glycogenolysis in the perfused liver [14–16]. Activators of the hepatic reticuloendothelial system (RES), such as particulates (latex beads and zymosan) and soluble aggregates of IgG, also increase hepatic glucose production transiently [17, 18]. Depolarization of the liver by elevated perfusate [K^+] stimulates glycogenolysis [19], as does infusion of adenine nucleotides and adenosine [20] and phorbol esters [21] in the perfused liver. However, these various non-hormonal substances do not stimulate glycogenolysis in either freshly isolated hepatic parenchymal cells or liver slices, in contrast with phenylephrine [16, 19–22]. The glycogenolytic response to autacoids, RES stimuli, [K^+], adenine nucleotides and phorbol esters parallels an agonist-induced vasoconstriction [14–21]. The present study was designed to determine whether organic trivalent arsenicals exert effects on certain specific physiological and pathophysiological glycogenolytic/vasoconstrictive agonists in the perfused liver.

Materials and methods

Liver perfusions and various measurements (oxygen consumption, portal pressure, and glucose production) were performed as described previously without modification [14, 23]. AGEPC and U-46619 were obtained from Bachem (Bubendorf, Switzerland) and Upjohn (Kalamazoo, MI) respectively. ATP was obtained from Pharmacia (Piscataway, NJ). L-Phenylephrine hydrochloride, prostaglandin E_2 (PGE_2) and adenosine were purchased from Sigma (St. Louis, MO). Vasopressin was acquired from Calbiochem (San Diego, CA). Phenylarsine oxide (PhAsO) and 2,3-dimercapto-1-propanol (BAL, British Anti-Lewisite) were purchased from Aldrich (Milwaukee, WI). 2-Mercaptoethanol (2-ME) was obtained from Bio-Rad (Richmond, CA). All other reagents were of the highest quality available from commercial suppliers.

Results and discussion

The α-adrenergic agonist, phenylephrine, stimulates glucose production and induces vasoconstriction in the perfused rat liver [24]. The dose–receptor relationships for phenylephrine-stimulated glycogenolysis and vasoconstriction in the presence and absence of PhAsO (10 μM) are shown in Fig. 1. In the absence of PhAsO, half-maximal increases in glucose production and portal pressure required 3×10^{-7} M and 2×10^{-6} M phenylephrine, respectively. At low physiological concentrations of phenylephrine (10^{-7} M), which do not constrict the hepatic vasculature, PhAsO had very little effect on phenylephrine-stimulated glycogenolysis. At higher concentrations of phenylephrine (10^{-6} to 10^{-5} M), which increased portal vein pressure markedly, PhAsO inhibited completely the hepatic hemodynamic response to phenylephrine while inhibiting phenylephrine-stimulated glycogenolysis by only 30% (Fig. 1). Thus, in the perfused liver, glucose output stimulated by high concentration of phenylephrine (10^{-5} M) may consist both of the well-documented direct

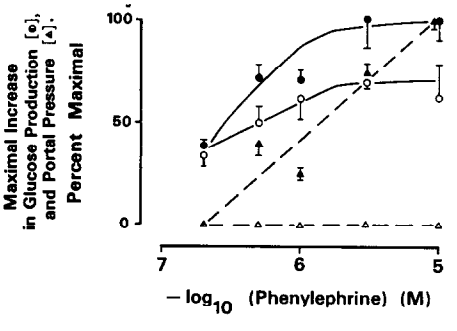

Fig. 1. Dose–response relationships of phenylephrine-stimulated hepatic glycogenolysis (circles) and vasoconstriction (triangles) in the absence (filled) and presence (open) of PhAsO (10 μM). PhAsO and phenylephrine were infused from 30 to 45 and 35 to 40 min of perfusion respectively. Portal pressure and glucose production were measured as described in Materials and Methods. The data are expressed as a percentage of the maximal stimulation obtained with 10 μM phenylephrine in the absence of PhAsO. Symbols and bars in the figure represent the mean and standard error for at least three separate determinations respectivly.

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Table 1. Effects of PhAsO (10 μM) on the hepatic vascular and glycogenolytic responses to various agonists

Agonist (conc)	Maximal change in agonist-stimulated: Glucose production (μmol·hr⁻¹·g⁻¹) −PhAsO	Glucose production (μmol·hr⁻¹·g⁻¹) +PhAsO	Portal pressure (mm Hg) −PhAsO	Portal pressure (mm Hg) +PhAsO
Phenylephrine (1 μM)	91 ± 2	79 ± 18	2.0 ± 0.1	ND*
Vasopressin (10 nM)	94 ± 10	97 ± 10	ND	ND
Adenosine (0.1 mM)	62 ± 5	57 ± 10	1.7 ± 0.6	0.6 ± 0.3
ATP (10 μM)	77 ± 16	35 ± 7	4.8 ± 0.6	0.8 ± 0.4
KCl (40 mM)	68 ± 14	27 ± 4	6.5 ± 1.0	1.7 ± 0.3
AGEPC (0.2 nM)	71 ± 4	16 ± 7	8.1 ± 1.0	0.8 ± 0.5
U-46619 (42 ng/ml)	92 ± 20	11 ± 1	13.8 ± 2.5	1.0 ± 0.4
PGE_2 (10 μM)	69 ± 19	37 ± 7	2.0 ± 0.6	ND

PhAsO and agonists were infused from 30 to 45 and 35 to 40 min of perfusion respectively. Values represent the mean ± SE for three to five separate determinations.
* ND: not detectable.

effects on parenchymal cells and indirect effects mediated by vasoconstriction-induced local ischemia. It would appear that PhAsO may selectively inhibit this latter process.

It seemed essential to extend these observations to other hepatic agonists, which probably exert their glycogenolytic effects either directly through interaction with parenchymal cells (e.g. glucagon and vasopressin) or indirectly through interaction with nonparenchymal cells (e.g. AGEPC, PGE_2 and U-46619). As shown in Table 1, PhAsO (10 μM) inhibited AGEPC-, U-46619-, PGE_2-, ATP-, and K^+-stimulated glycogenolysis by 50–80%. Vasoconstriction stimulated by these agonists was inhibited similarly by PhAsO. In contrast, phenylephrine-, vasopressin- and adenosine-stimulated glycogenolysis were inhibited less than 15% by PhAsO. However, phenylephrine- and adenosine-stimulated hepatic vasoconstriction were inhibited substantially by PhAsO. Vasopressin did not cause hepatic hemodynamic effects. Glucagon (2 nM)- and isoproterenol (10 μM)-stimulated glycogenolysis are not inhibited by PhAsO (10 μM) infusion, although immune-aggregate-stimulated glycogenolysis and vasoconstriction are inhibited by arsenicals [23].

We examined the ability of thiol compounds (2-ME or BAL) to reverse PhAsO inhibition of phenylephrine-induced vasoconstriction and glycogenolysis (Table 2). Infusion of 2-ME at 40-fold molar excess relative to arsenical did not reverse the effects of PhAsO. In contrast, a near-stoichiometric concentration of BAL was able to restore phenylephrine-stimulated vasoconstriction. The relatively minor inhibition by PhAsO of phenylephrine-stimulated glycogenolysis was restored similarly by BAL infusion (Table 2). These findings suggest that vicinal dithiols are involved in the hemodynamic responsiveness of the hepatic vasculature to phenylephrine and other vasoconstrictors, such as immune-aggregates [23].

It is noteable that those agonists (AGEPC, PGE_2, U-46619, immune-aggregates, K^+) which appear to act through the sinusoidal cells (Kupffer and endothelial) of the liver share sensitivity to PhAsO. In contrast, agonists which act directly on parenchymal cells to stimulate gly-

Table 2. Effects of thiols on PhAsO inhibition of phenylephrine-stimulated vasoconstriction and glycogenolysis

Treatment	Phenylephrine (10 μM)-stimulated maximal change in: Glucose production (μmol·hr⁻¹·g⁻¹)	Portal pressure (mm Hg)
Vehicle	161 ± 8	5.5 ± 1.2
PhAsO (10 μM)	116 ± 16	ND*
2-ME (0.4 mM), PhAsO (10 μM)	101 ± 7	ND
BAL (30 μM), PhAsO (10 μM)	140 ± 3	4.3 ± 1.3

Thiols (2-ME or BAL) and PhAsO (or ethanol as vehicle) were infused from 35 to 42.5 and 42.5 to 50 min of perfusion respectively. All livers were infused with phenylephrine from 50 to 55 min of perfusion. Values in the table represent the mean ± SE for three separate determinations.
* ND: not detectable.

cogenolysis (i.e. phenylephrine, isoproterenol, glucagon, and vasopressin) are relatively insensitive to arsenicals. These findings are consistent with the proposal of Datta *et al.* [12] that trivalent arsenicals can alter the function of the hepatic vascular endothelium. Furthermore, higher doses of PhAsO (100 μM) alone increased substantially and irreversibly (over 30 min) portal vein pressure (0.9 ± 0.3 mm Hg) and glucose production (80 ± 15 μmol·hr^{-1}·g^{-1}).

The precise mechanism(s) by which trivalent arsenicals inhibit autacoid-mediated hepatic responses remains unknown. However, the close correlation between hepatic vasoconstriction and glycogenolysis found for several autacoids suggests that vasoconstriction-induced ischemia increases glycogenolysis in these situations [16, 19–21]. In such a scenario, trivalent arsenicals would inhibit hepatic glycogenolysis primarily by preventing agonist-induced vasoconstriction. Although arsenical toxicity has been attributed generally to their energy-depleting action [1], we observed effects on hepatic autacoid-stimulated vasoconstriction and glycogenolysis at arsenical doses which have only minor effects on hepatic oxygen consumption and which do not alter hepatic adenine nucleotide concentrations [23]. Thus, a primary locus for arsenical action may be proteins involved in the coupling of agonist binding with various cellular responses. Although the nature of such proteins remains unknown, Bernier *et al.* [25] have identified a 15,000 kD protein whose phosphorylation increases specifically when cells are stimulated with hormone (insulin) in the presence of PhAsO. Therefore, it seems appropriate to speculate that a major result of toxic arsenical exposure is a diminished responsiveness of cells to various autacoid signals. Such cellular desensitization may participate in the development of the pathological lesions that characterize arsenical toxicosis.

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Presence of the toxic metabolite *N*-hydroxy-norcocaine in brain and liver of the mouse

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The appearance of *N*-hydroxy-norcocaine (NHNC) in the liver has been associated with the hepatotoxic effect of cocaine [1]. The hepatotoxicity arises with production of a nitroxide free radical, a product of NHNC. This metabolic pathway is associated with the microsomal cytochrome P-450 system [2]. The metabolic sequence whereby cocaine is demethylated to norcocaine and then metabolized to NHNC and to a nitroxide free radical is active in the liver [2, 3]; however, the presence, or metabolic formation, *in vivo*, of NHNC in the central nervous system has not been established. Conversion of cocaine to norcocaine in rat brain after intracisternal injection has been reported [4], and the presence of norcocaine in brain after i.v. or s.c. administration of cocaine in rats has been demonstrated [5]. Since this pathway is toxic in liver, it was of interest to determine if NHNC is also present in brain.