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# Arylacetamide Deacetylase Activity Towards Monoacetyldapsone

## SPECIES COMPARISON, FACTORS THAT INFLUENCE ACTIVITY, AND COMPARISON WITH 2-ACETYLAMINOFLUORENE AND *p*-NITROPHENYL ACETATE HYDROLYSIS

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**ABSTRACT.** The deacetylation of monoacetyldapsone (MADDS) was examined in liver microsomes and cytosol from male Sprague–Dawley rats, Golden Syrian hamsters, and Swiss Albino mice. All three rodent species demonstrated greater MADDS deacetylation activity in liver microsomes than in liver cytosol. Further investigations were conducted in hamsters. The velocity of MADDS deacetylation in major organs in the hamster was greatest in the intestine, followed by the liver and kidney. The effect of pretreatment with common inducers on liver microsomal deacetylation activity was also examined in the hamster. Phenobarbital, 100 mg/kg/day × 3 days, did not alter activity, while dexamethasone at the same dose reduced 2-acetylaminofluorene (2-AFF), MADDS, and *p*-nitrophenyl acetate (NPA) hydrolysis by at least 50%. Due to a previous report that KI activated the deacetylation of an arylacetamide *in vitro* (Khanna *et al.*, *J Pharmacol Exp Ther* **262:** 1225–1231, 1992), the effects of the halides KF, KCl, KBr and KI on MADDS hydrolysis *in vitro* were tested. Of the halides studied, only KF altered MADDS hydrolysis, resulting in an almost complete inhibition of deacetylase activity at 50 mM (with the initial concentration of MADDS at 0.6 mM) with an $IC_{50}$ = 0.16 mM. Cornish–Bowden and Dixon plots indicated that the inhibition exerted by KF was non-competitive. The rank order of inhibitor potencies was constructed using phenylmethylsulfonyl fluoride (PMSF), bis(*p*-nitrophenyl)phosphate (BNPP), physostigmine, and KF with 2-AFF, MADDS, and NPA as substrates. Different rank order potencies were obtained for each of the substrates tested. The substrates 2-AFF, MADDS, and NPA did not act as competitive inhibitors on the hydrolysis rates of each other. Liver microsomal arylacetamide deacetylase activity was greater in male hamsters than in females with either MADDS or 2-AAF as substrates; however, hydrolysis of NPA was similar in both male and female hamsters. These data support the hypothesis that the enzyme which catalyzes the hydrolysis of MADDS differs from that catalyzing either 2-AAF or NPA hydrolysis. BIOCHEM PHARMACOL 51;12:1661–1668, 1996.

**KEY WORDS.** 2-acetylaminofluorene; arylacetamide deacetylase; dapsone; deacetylation; hamsters; liver microsomes; monoacetyldapsone; *p*-nitrophenyl acetate

Exposure to arylamines is associated with a variety of toxicities. Numerous studies have demonstrated that the ability of subjects to acetylate arylamine compounds (forming arylacetamides) is an important predisposing factor in the development of toxicity from these agents [1–3]. It is important, however, to recognize that acetylation of arylamines is not an irreversible process. Numerous arylacetamides have been shown to undergo deacetylation *in vivo* and *in vitro* [4–6]. Hence, the relative rate of acetylation and deacetylation may be the more relevant parameter to assess when attempting to associate arylamine disposition with the risk of toxicity. Indeed, some experimental evidence indicates that hydrolysis of arylacetamides is a critical step in arylamine-induced carcinogenesis [7].

To date, however, little is known about the enzyme(s) that catalyzes the hydrolysis of arylacetamides (which we refer to herein as ADA†). In our initial studies to characterize the relevant enzyme(s), we have examined the potential utility of MADDS as a probe for ADA. MADDS is the monoacetylated metabolite of DDS, an agent used in the treatment of leprosy, dermatitis herpetiformis, and *Pneumocystis carinii* pneumonia in patients with AIDS [8–10]. The studies described herein sought to test the hypothesis that the deacetylation of MADDS is catalyzed by a currently uncharacterized and unidentified enzyme.

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† *Abbreviations:* ADA, arylacetamide deacetylase; 2-AF, 2-aminofluorene; 2-AAF, 2-acetylaminofluorene; BNPP, bis(*p*-nitrophenyl)phosphate; DDS, dapsone; MADDS, monoacetyldapsone; NP, *p*-nitrophenol; NPA, *p*-nitrophenyl acetate; PMSF, phenylmethylsulfonyl fluoride; SMZ, sulfamethazine; and NAT, *N*-acetyltransferase.

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## MATERIALS AND METHODS

### Materials

MADDS and DDS were gifts from Parke–Davis (Ann Arbor, MI). Sodium SMZ, KF, PMSF, BNPP sodium salt, and dexamethasone-21-phosphate disodium were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were obtained from commercial vendors and used as received.

### Animals

Male and female Golden Syrian hamsters weighing 80–100 g, male Swiss Albino mice weighing 25–35 g, and male Sprague–Dawley rats weighing 150–175 g were purchased from Charles River Breeding Laboratories (Wilmington, MA). The animal species were housed separately in plastic cages containing wood bedding in a temperature- and humidity-controlled environment until used. Animals had free access to laboratory chow and water *ad lib.* until they were killed.

### Tissue Preparation

The liver (or kidney and intestine) was removed surgically (the intestine was flushed with normal saline to remove luminal contents) after $CO_2$ euthanasia, weighed, minced, and homogenized in ice-cold Sorensen's phosphate buffer (pH 7.4) with a Teflon pestle. The homogenate was centrifuged at 10,000 *g* for 10 min, after which the corresponding supernatant was decanted into another centrifuge tube. This supernatant was centrifuged at 100,000 *g* for 1 hr, with the resulting supernatant used as the source of cytosol and the pellet used as the source of microsomes. The microsomal pellet was resuspended in ice-cold Sorensen's phosphate buffer via a Teflon pestle. Unused portions of cytosol or microsomes were stored at −70° until further use.

### Determination of Enzyme Activity

Cytosolic or microsomal ADA activity was determined primarily using MADDS as the substrate. Aliquots of cytosol or microsomes (200 μL) and Sorensen's phosphate buffer (250 μL) were preincubated for 5 min at 37° in a temperature-controlled shaker bath. The reaction was started by the addition of 50 μL of MADDS (final concentration of 0.6 mM, unless otherwise stated) and incubated for 60 min, following which the reaction was stopped by the addition of 200 μL of cold methanol. Preliminary studies indicated that DDS formation was linear for up to 180 min and with initial concentrations of MADDS ranging from 0.075 to 1.8 mM (data not shown). Linearity as a function of subcellular fraction protein content was also demonstrated. The concentration of the deacetylated metabolite was determined as described below. Incubations were performed in triplicate and expressed as nanomoles of DDS formed per hour per milligram of protein. Protein concentrations were determined utilizing the microprotein determination method of Bradford [11]. Halides, PMSF, BNPP, physostigmine, 2-AAF, and NPA (at concentrations specified in results) were preincubated with hamster microsomes for 10 min at 37° prior to the addition of MADDS. ADA activity was then determined as previously described.

In some experiments, hydrolytic activity towards 2-AAF and NPA was also determined. For 2-AAF, incubations were conducted as described for MADDS, except that the reaction was started by the addition of 50 μL of 2-AAF (final concentration of 0.6 mM, unless otherwise stated), was incubated for 10 min, and was stopped by the addition of 500 μL of cold methanol. NPA was dissolved in 10% DMSO in Sorensen's phosphate buffer (pH 7.4) sufficient to provide a concentration of 1 mM. Three milliliters of this solution was placed in a cuvette, to which 5 μL of microsomes was added and the absorbance of the incubation followed for 3 min.

### Assay of DDS

DDS concentrations were determined using the HPLC method described by Coleman *et al.* [12]. To each sample, 75 μL of 0.6 mM sodium SMZ (internal standard) and 3 mL of water-saturated ethyl acetate were added, followed by mixing on a rotomixer and then centrifuged (500 *g*) for 10 min. The organic layer was removed and evaporated to dryness under a steady stream of $N_2$ and mild heat (58°), followed by reconstitution in 200 μL of mobile phase. A 10-μL aliquot was injected onto a 10 μm $C_{18}$-μBondapak® column (Waters Associates, Milford, MA). Elution was accomplished using a mobile phase of 400:100:5:0.25 of water:acetonitrile:glacial acetic acid:triethylamine at a flow rate of 1.2 mL/min, and detection was performed using a fixed-wavelength UV detector with a 254 nm filter (Waters Associates model 441). Concentrations were quantified from a standard curve generated with spiked samples of known concentration using the peak area ratio of DDS:SMZ determined on a Hewlett Packard 3396A integrator (Novi, MI). The retention times were 7.71, 12.6, and 16.8 min for SMZ, DDS, and MADDS, respectively.

### Assay of 2-AF

2-AF concentrations were determined using the HPLC method described by Probst *et al.* [13], with minor modifications. To each sample, 100 μL of 0.06 mM 2-hydroxycarbazole (internal standard) was added and followed by mixing on a rotomixer and then centrifuged (500 *g*) for 5 min. A 50-μL aliquot of the supernatant was injected onto a 10 μm $C_{18}$-μBondapak® column (Waters Associates). Elution was accomplished using a mobile phase of 20 mM $NaClO_4$ in water:acetonitrile (61:39) at a flow rate of 2 mL/min, while detection was performed using a fixed-wavelength UV detector with a 254 nm filter (Waters Associates model 441). Concentrations were quantified from a standard curve generated with spiked samples of known concentration using a peak area ratio of 2-AF:hydroxy-

carbazole determined on a Hewlett Packard 3396A integrator. The retention times were found to be 6.0, 8.5, and 10.2 min for 2-hydroxy-carbazole, 2-AAF, and 2-AF, respectively.

### *Assay of NPA*

The hydrolysis of NPA was determined using the assay of Krisch [14]. After the addition of the tissue fraction to the NPA solution, the sample cuvette was quickly inverted three times, following which absorbance was monitored at 405 nm for 3 min. The amount of NP formed was determined as

$$\Delta A/\varepsilon l \times 10^9 \times 0.003 \text{ L} = \text{nmol formed/min}$$

where $\Delta A$ = change in absorbance per minute, $\varepsilon$ = molar absorptivity (16,400 L/min/mol) and $l$ = cell path length. The amount formed per minute was normalized to protein content.

### *Animal Treatment*

Hamsters received daily i.p. injections of phenobarbital or dexamethasone dissolved in sterile water at a dose of 100 mg/kg/day × 3 days. A control group, which received an equivalent volume of sterile water, was studied in parallel. Animals were killed 24 hr after the last dose, liver was removed and a microsomal pellet was obtained as described previously.

### *Data Analysis*

Identification of discordant data in repeated determinations of DDS formation rates in all experiments was made by the Q test [15]. Characterization of the inhibition of ADA by KF was conducted by constructing double-reciprocal Dixon and Cornish–Bowden plots [16]. Best-fit lines for each graph were determined using least squares regression analysis. Statistical comparison of hydrolysis activity in the presence and absence of various inducers was conducted using one-way analysis of variance with the Student–Newman–Keuls test for multiple comparisons. Statistical comparison of hydrolysis between male and female hamsters was conducted using the $t$-test. A value of $P < 0.05$ was considered as significant.

## RESULTS

### *Comparison of MADDS Hydrolysis in Different Species and Tissues*

The velocity of DDS formation in liver cytosol and microsomes from different male rodents is shown in Table 1. Microsomal ADA activity was greatest in the mouse followed by the hamster and then the rat. ADA activity in liver cytosol was consistently less than one order of magnitude of the activity in the microsomal fraction.

The velocity of DDS formation in liver, kidney, and intestinal microsomes from male hamsters is compared in Table 2. When normalized to microsomal protein content, both the liver and intestinal microsomes demonstrated one order of magnitude greater hydrolysis activity than the kidney microsomes. Intestinal microsomes demonstrated twice the activity observed in liver microsomes.

**TABLE 1. Species comparison of MADDS hydrolysis activity in the liver**

| Species | DDS Formation (nmol/hr/mg protein) | |
|---|---|---|
| | Microsomes | Cytosol |
| Rat | 1.45 ± 0.79 | 0.15 ± 0.04 |
| Hamster | 36.1 ± 4.9 | 2.14 ± 0.75 |
| Mouse | 113 ± 11 | 1.54 ± 0.27 |

MADDS hydrolysis was measured in 60-min incubations with 0.6 mM MADDS. Values are means ± SD for 4 male animals in each species.

### *Effect of Potential Modulators of Hydrolysis Activity*

The effects of common inducers of microsomal enzymes on 2-AAF, MADDS, and NPA hydrolysis in hamster liver microsomes are shown in Table 3. Pretreatment of animals with phenobarbital did not alter hydrolysis activity significantly as compared with control, whereas dexamethasone decreased hydrolysis activity by at least 50% as compared with phenobarbital and control.

Khanna *et al.* [17] have reported that KI activated thiacetazone *N*-deacetylase *in vitro*. Therefore, a series of halides was examined for their potential modulation of ADA activity. As shown in Fig. 1, KCl, KBr and KI did not demonstrate any effect on ADA activity. KF significantly inhibited ADA activity towards MADDS with an $IC_{50}$ = 0.16 mM. To determine if the alkali metals exert any effect in this inhibition, KF and NaF effects were compared. As shown in Fig. 2, there was no difference between KF and NaF inhibition in the concentration range of 0.05 to 1 mM. Cornish–Bowden and Dixon plots were constructed using MADDS initial incubation concentrations of 0.6 and 0.3 mM (Fig. 3). These plots suggest that KF is a noncompetitive inhibitor of ADA.

Rank inhibition studies were done with BNPP, KF, physostigmine, and PMSF as inhibitors with NPA, MADDS,

**TABLE 2. Comparison of microsomal MADDS hydrolysis activity in various tissues from the hamster**

| Tissue | DDS Formation (nmol/hr/mg protein) |
|---|---|
| Liver | 43.7 ± 7.5 |
| Intestine | 86.0 ± 30.4 |
| Kidney | 4.4 ± 0.6 |

MADDS hydrolysis was measured in 60-min incubations with 0.6 mM MADDS. Values are means ± SD for 4 male animals in each tissue.

**TABLE 3. Comparisons of hydrolytic activity towards various substrates in liver microsomes from male hamsters treated with microsomal enzyme inducers**

| Treatment | Hydrolysis activity | | |
|---|---|---|---|
| | MADDS (nmol DDS formed hr/ mg protein) | 2-AAF (nmol 2-AF formed/min/ mg protein) | NPA (μmol NP formed/min/ mg protein) |
| Control | 24.3 ± 3.96 | 3.28 ± 0.60 | 6.61 ± 1.28 |
| Phenobarbital | 27.8 ± 2.22 | 3.52 ± 0.65 | 6.34 ± 0.89 |
| Dexamethasone | 8.98 ± 0.49* | 1.40 ± 0.17* | 2.25 ± 0.23* |

Animals were pretreated with sterile water (control), phenobarbital, or dexamethasone, 100 mg/kg/day for 3 days, prior to removal of liver. Values are means ± SD, N = 4.

* $P < 0.05$ dexamethasone vs control and dexamethasone vs phenobarbital.

and 2-AAF as substrates (Fig. 4). While solubility limits prevented achievement of >50% inhibition for some compounds, the data suggest that a rank of inhibition potency would be the following: KF > BNNP > physostigmine = PMSF, BNPP > PMSF > physostigmine > KF, and BNPP > physostigmine > PMSF > KF for NPA, MADDS, and 2-AAF, respectively. In separate experiments, competition studies were done with 2-AAF, MADDS, and NPA utilizing the other two substrates as potential competitive inhibitors (Table 4). The data suggest that the substrates do not compete with each other as substrates for hydrolysis.

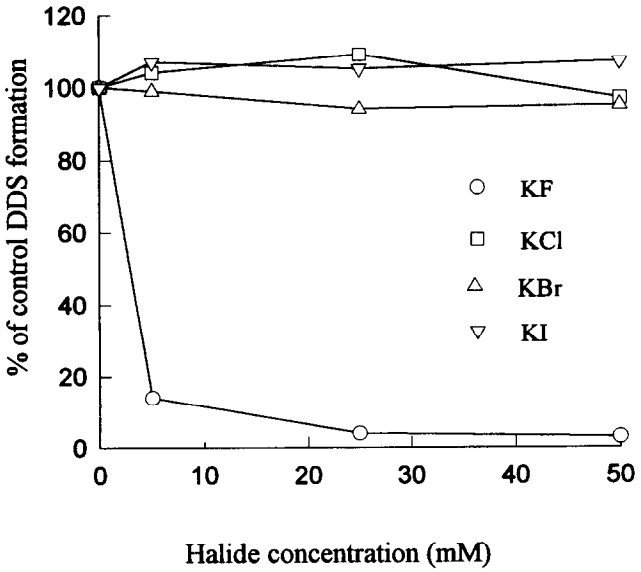


**FIG. 1. Effect of halides on MADDS hydrolysis in hamster liver microsomes. MADDS hydrolysis in the presence and absence of halides at concentrations of 5, 25 and 50 mM was determined with initial MADDS concentrations of 0.6 mM. Data are presented as mean values of pooled male microsomes incubated in triplicate. Control velocity was 150 nmol of DDS formed/hr.**

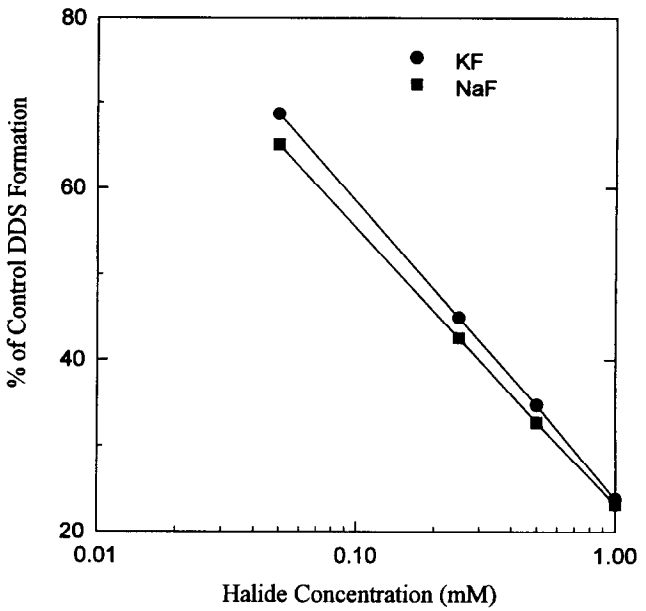


**FIG. 2. Effect of KF and NaF on MADDS hydrolysis. Incubations were conducted in pooled male hamster liver microsomes incubated in triplicate with initial MADDS concentrations of 0.6 mM. Each data point represents the mean value. The lines are regression lines. Incubations at various inhibitor concentrations that exhibited >80% or <20% control activity were excluded from the graph. Control velocity was 250 nmol of DDS formed/hr.**

### *Sex Dependence and Comparison Activity Towards Substrates*

The activity towards MADDS, 2-AAF, and NPA in male and female hamster liver microsomes is shown in Table 5. Male hamsters demonstrated significantly greater ADA activity with MADDS and 2-AAF as substrates than did the females. Activity in females towards MADDS and 2-AAF was 31 and 23% lower, respectively. However, there was no significant difference in the hydrolysis of NPA in liver microsomes from male and female hamsters. The activities toward NPA and 2-AAF were substantially greater than that towards MADDS.

## DISCUSSION

While deacetylation has been shown to be an important step in the bioactivation of arylamine carcinogens [7], little is known about the enzyme(s) which catalyzes this reaction. Early work by Irving [5] demonstrated the ability of liver microsomes from several species to deacetylate *N*-hydroxy-2-acetylaminofluorene. This investigator found the greatest activity towards this substrate in the guinea pig, followed by the rabbit and then the rat. Other investigators have also found deacetylase activity towards this and other substrates in hamsters, mice, and dogs [18–20]. Of the therapeutic agents and their metabolites studied to date, MADDS appears to be the arylacetamide most extensively deacetylated in humans [6]. This suggested that MADDS may be a useful

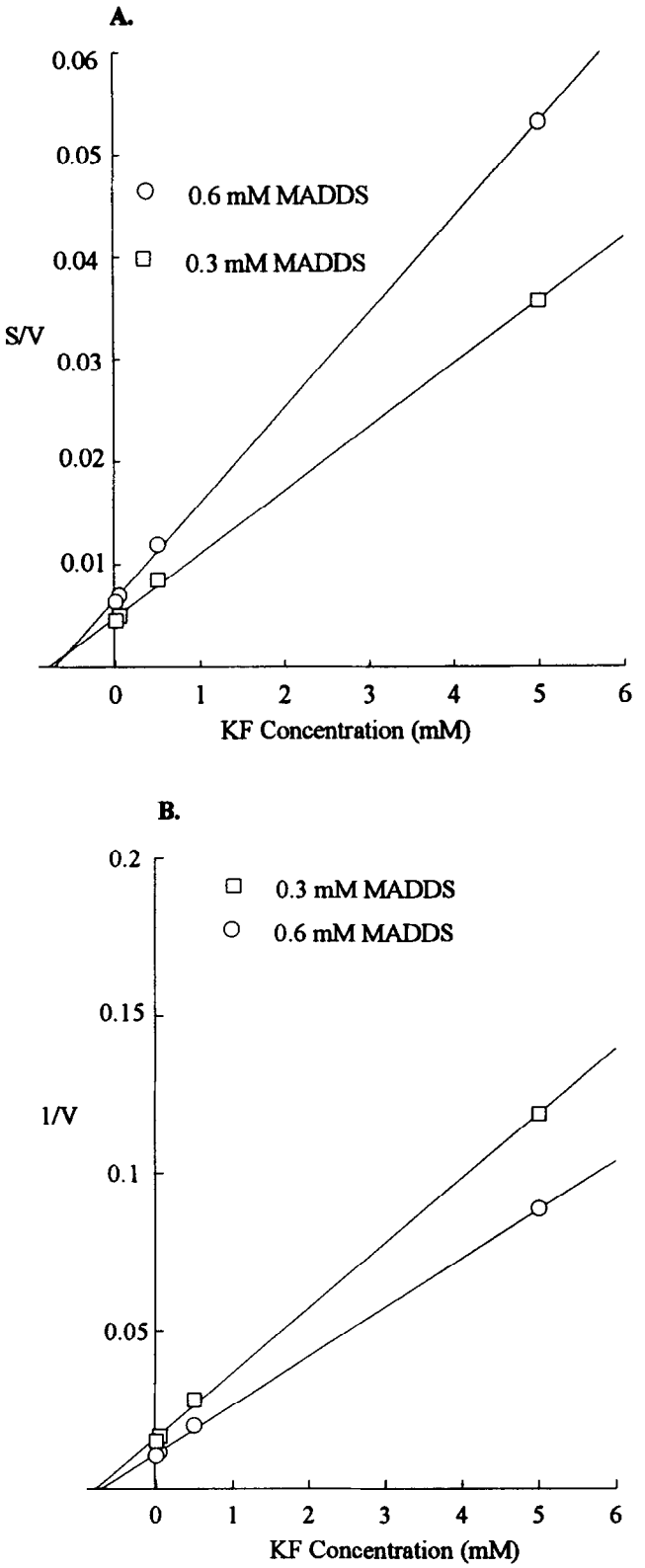


**FIG. 3. Cornish–Bowden and Dixon plots of the effect of KF on ADA activity towards MADDS. (A) Cornish–Bowden plot. (B) Dixon plot. Inhibition of ADA activity by KF was studied with initial MADDS concentrations as shown. Each point represents the mean of three determinations in pooled male hamster liver microsomes, while the line was derived from least squares regression.**

primary probe for identifying and characterizing the enzyme responsible for arylacetamide deacetylase.

In the present study, MADDS deacetylation was found to be greatest in the microsomes, and the activity towards this substrate in the three species examined was found to be mouse > hamster > rat. We decided to conduct further studies using the hamster, since there is a hamster strain congenic at the *NAT2* locus which may prove useful in assessing the role of acetylation/deacetylation in arylamine toxicity [21, 22].

When comparing the activity of MADDS hydrolysis in various tissues, activity was found to be greatest in the intestine, followed by the liver and then the kidney. This contrasts with the observations of Irving [5], who found that guinea pig liver homogenates exhibited activity at least ten times greater than any other tissue homogenate. The differences in tissue ADA activity in the present study and that by Irving may be due to species differences or differences in substrates. In the present study, significantly greater variability was observed in the intestinal activity than in hepatic or renal activity. This is probably secondary to the loss of cells from the intestinal lumen during the flushing of its contents, a step that is unnecessary with hepatic or renal tissue.

The identity and potential multiplicity of enzymes catalyzing arylacetamide deacetylation remain unresolved issues. Several investigators have suggested that the enzyme responsible for deacetylation of arylacetamides is a carboxylesterase [23–25]. Kaur and Ali [26] have demonstrated that rat liver microsomal esterase activity toward acetylsalicylic acid, procaine, and NPA were all induced by phenobarbital pretreatment, as was the deacetylation of acetanilide and 2-AAF. This suggests common regulation of carboxylesterase activity and 2-AAF deacetylation. However, in the present investigation, phenobarbital had no effect on 2-AAF, MADDS, or NPA hydrolysis. In contrast, dexamethasone resulted in at least 50% reduction in liver microsomal 2-AAF, MADDS, and NPA hydrolysis activity. Interestingly, dexamethasone pretreatment has also been found to result in a substantial reduction in NPA hydrolysis in rat liver microsomes [27]. This observation suggests that the enzyme(s) responsible for the hydrolysis of the three substrates is(are) down-regulated by dexamethasone.

Khanna *et al.* [17] have demonstrated that NaI and KI activated cytosolic ADA activity towards the arylacetamide thiacetazone *in vitro*. Though none of the halides tested increased ADA activity for MADDS, KF and NaF did decrease ADA activity in a similar fashion. The type of inhibition exerted by KF was determined by constructing Dixon and Cornish–Bowden plots. These two plots were used because they demonstrate different graphical representations for mechanisms of enzymatic inhibition and thus facilitate identification of the type of inhibition [16]. The results suggested that the type of inhibition by KF was noncompetitive. As NaF and KF are shown to inhibit carboxylesterases, this latter observation suggested that MADDS

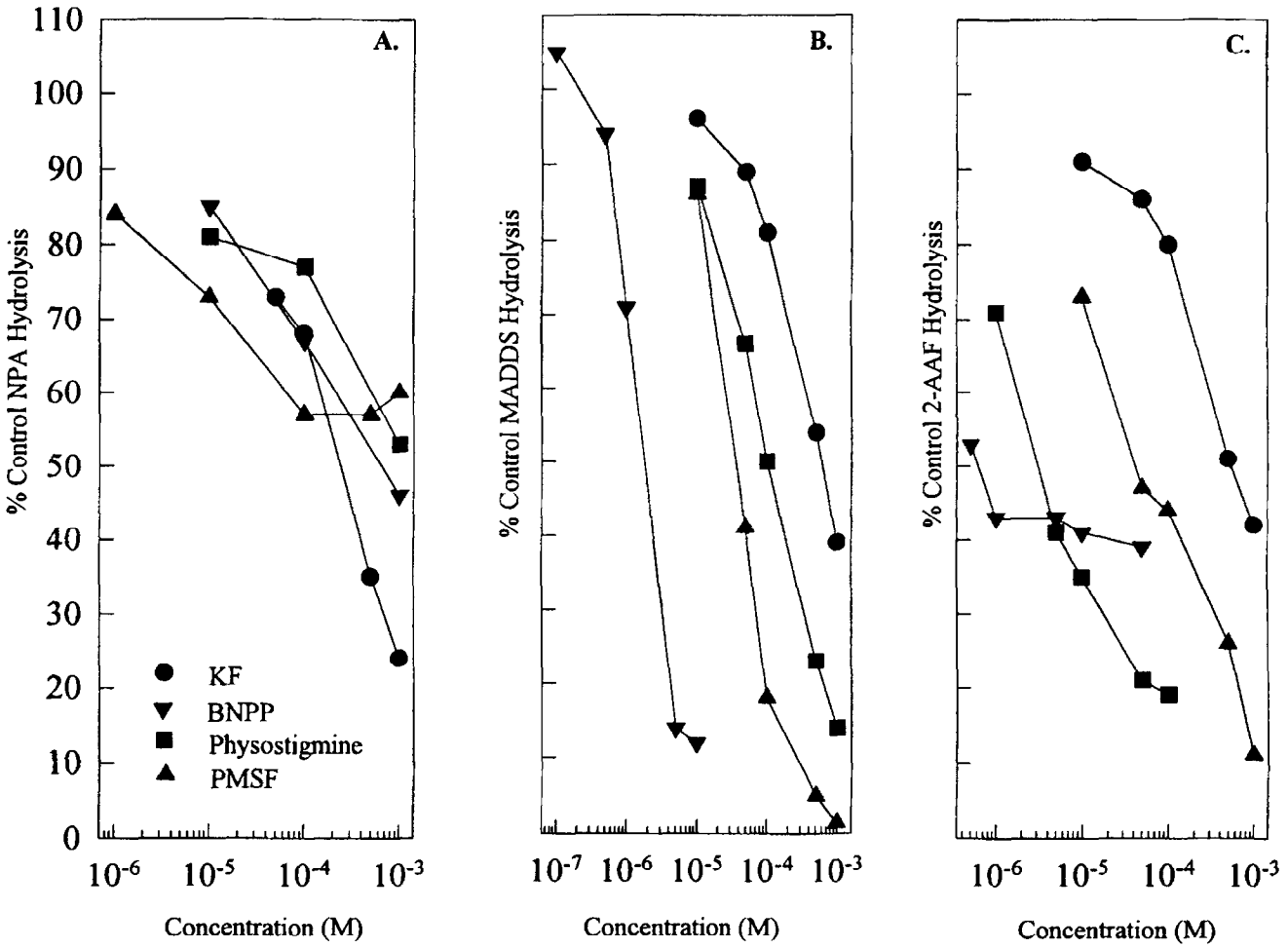


**FIG. 4. Effect of BNPP, KF, physostigmine, and PMSF on NPA (A), MADDS (B), and 2-AAF (C) hydrolysis. Inhibition of hydrolysis activity by BNPP, KF, physostigmine, and PMSF was studied with an initial NPA concentration of 1 mM, an MADDS concentration of 0.6 mM, and a 2-AAF concentration of 0.6 mM. Each point represents the average of two determinations in male liver microsomes. Control velocities were 78 μmol of NP formed/min, 65 nmol of DDS formed/hr, and 26 nmol of 2-AF formed/min for NPA, MADDS, and 2-AAF, respectively.**

deacetylation may be catalyzed by a common carboxylesterase.

Several studies were conducted in an effort to determine if MADDS, 2-AAF, and NPA hydrolyses were catalyzed by the same enzyme. First, inhibition studies were conducted using BNPP, physostigmine, PMSF, and KF, which have been reported previously to inhibit either 2-AAF or NPA hydrolysis [25, 28]. NPA hydrolysis is more sensitive to KF, whereas MADDS and 2-AAF hydrolysis are more sensitive to BNPP. This suggests that a different enzyme catalyzes the hydrolysis of NPA as compared with MADDS and 2-AAF. Also, MADDS and 2-AAF exhibited a different rank order for the inhibition examined, which suggests that a different enzyme catalyzes the hydrolysis of MADDS as compared with 2-AAF.

Several interesting observations arose from the inhibition studies. First, as shown in Fig. 4A, inhibition of NPA hydrolysis by PMSF suggested the possibility of enzyme multiplicity. Approximately 50% of NPA hydrolysis was inhibited as the concentration of PMSF was increased from $10^{-6}$ to $10^{-4}$ M. Further increases in PMSF to the limit of solubility did not result in further inhibition. This suggests that NPA hydrolysis is catalyzed by at least two enzymes, one more sensitive to PMSF inhibition than the other. Indeed, Morgan *et al.* [29] have reported the purification of two microsomal carboxylesterases from rat liver microsomes, designated hydrolases A and B. Hydrolase A was found to be PMSF-sensitive ($IC_{50}$ = 100 nM), while hydrolase B was PMSF-insensitive. The observations made in the present investigation are consistent with a similar multiplicity in hamster liver microsomal carboxylesterase activity. Similarly, while a concentration of $10^{-6}$ M BNPP was sufficient to cause >50% inhibition of 2-AAF deacetylation, further increases in the concentration up to $10^{-4}$ M did not cause further inhibition. This observation is consistent with multiple enzymes being responsible for 2-AAF deacetylation, one of which is BNPP-sensitive and the other BNPP-insensitive. In contrast, inhibition curves con-

**TABLE 4. Hydrolysis inhibition studies using NPA, 2-AAF, and MADDS as competitive inhibitors in pooled male liver microsomes**

| Competitive inhibitor | Hydrolysis activity | | |
|---|---|---|---|
| | NPA (μmol NP formed/min/ mg protein) | 2-AAF (nmol 2-AF formed/min/ mg protein) | MADDS (nmol DDS formed/hr/ mg protein) |
| Control | 2.05 | 1.09 | 2.72 |
| MADDS 0.6 mM | 2.73 | 0.873 | |
| MADDS 0.3 mM | 2.73 | 0.795 | |
| MADDS 0.1 mM | 2.30 | 1.07 | |
| 2-AAF 0.6 mM | -* | | 2.43 |
| 2-AAF 0.3 mM | -* | | 2.78† |
| 2-AA 0.1 mM | 2.05 | | 2.70† |
| NPA 0.6 mM | | 1.00 | 3.06 |
| NPA 0.3 mM | | 1.32 | 3.31 |
| NPA 0.1 mM | | 1.79 | 3.00 |

The final incubation concentration of measured substrates was 0.1 mM. Measurements were performed in duplicate.

* Hydrolysis could not be determined because of assay interference.

† One measurement.

sistent with a single enzyme (or multiple enzymes with similar sensitivity) were seen for all inhibitors with MADDS deacetylation.

If the three substrates examined are hydrolyzed by a common enzyme, they should be mutually inhibitory. Competitive inhibition studies with 2-AAF, MADDS, and NPA found that these compounds do not inhibit each other's rate of hydrolysis, which suggests that these compounds are hydrolyzed by different enzymes. This conclusion assumes that the concentrations of substrates used were above the $K_m$. This is clearly the case for MADDS ($K_m$ = 0.3 mM) and assumed for 2-AAF and NPA.

Rat liver microsomal carboxylesterase activity has been shown to display sex-dependent expression [30]. NPA hydrolysis in male liver microsomes is greater than that observed in female rat liver microsomes. To examine for further regulatory commonalities for the substrates examined, hydrolysis of the three substrates was examined in liver microsomes for adult male and female hamsters. In contrast to observations made in the rat, sex-dependent NPA hydrolysis was not observed. However, activity towards MADDS and 2-AAF was higher in male hamster liver microsomes than in those obtained from females. This observation provides further evidence that MADDS and 2-AAF deacetylation are catalyzed by enzymes distinct from classic nonspecific carboxylesterases.

**TABLE 5. Comparison of activity towards various substrates in liver microsomes from male and female hamsters**

| Sex | Hydrolysis activity | | |
|---|---|---|---|
| | MADDS (nmol DDS formed/hr/ mg protein) | 2-AAF (nmol 2-AF formed/min/ mg protein) | NPA (μmol NP formed/min/ mg protein) |
| Male | 22.9 ± 1.14* | 4.75 ± 0.37* | 4.41 ± 0.91 |
| Female | 15.8 ± 2.07 | 3.67 ± 0.47 | 4.80 ± 0.89 |

Values are means ± SD, N = 4.

* $P < 0.05$ male vs female.

Recently, Probst *et al.* reported the purification [13] and subsequent cloning [28] of a human liver microsomal enzyme that catalyzes the deacetylation of 2-AAF. The N-terminal sequence of this protein is distinct from that of any known carboxylesterase. Further analysis resulted in the conclusion by these investigators that the human 2-AAF deacetylase was appropriately classified as an esterase.

In summary, the present investigations indicate that MADDS is hydrolyzed primarily by a microsomal enzyme in hamster liver. The activity of the enzyme is down-regulated by dexamethasone pretreatment, as is the activity towards 2-AAF and NPA. Similar to 2-AAF but in contrast to NPA, MADDS hydrolysis displays sex-dependent activity. These results, combined with inhibition experiments, indicates that a common carboxylesterase is not the primary enzyme that catalyzes the deacetylation of MADDS. It would also appear that MADDS deacetylation is catalyzed by an ADA distinct from that which catalyzes 2-AAF deacetylation. Purification of the enzyme(s) and subsequent characterization will be necessary to assess the potential multiplicity of ADA and its appropriate classification. These investigations are currently in progress.

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