Journal of Chromatography, 430 (1988) 123–127
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4243

Note

Species-dependent differences in recovery of 3,4-dihydroxybenzylamine in assays of plasma catecholamines

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(First received February 12th, 1988; revised manuscript received March 24th, 1988)

3,4-Dihydroxybenzylamine (DHBA) frequently has been used as an internal standard in assays of plasma catecholamines, since analytical recoveries of DHBA have been the same as those of endogenous catecholamines in plasma of humans, monkeys, pigs, rats, mice and fetal sheep [1]. In dogs, cattle, horses, and newborn and adult sheep, however, recoveries of DHBA have been much smaller than those of endogenous catecholamines [1–3]. This would result in erroneously high estimates of catecholamine levels. No experimental information is available about the cause of this phenomenon.

Black bedouin goats, animals inhabiting the desert, have been used in studies of homeostatic responses of vasopressin and the renin–angiotensin–aldosterone system during hypovolemia [4–6]. Since the sympathoadrenomedullary system participates in regulation of blood volume and pressure, we recently have been measuring plasma levels of catecholamines in dehydrated bedouin goats. For these measurements we have used alumina extraction followed by liquid chromatography with electrochemical detection (LC–ED). We report here that recoveries of DHBA were much smaller than those of the endogenous catecholamines norepinephrine (NE) and epinephrine (E). The recoveries increased when the samples were deproteinized before the alumina extraction step.

EXPERIMENTAL

Materials

The chromatographic apparatus consisted of a Varian 5000 liquid chromatograph, Bioanalytical Systems LC-4A electrochemical detector, Bioanalytical Systems LC-17 oxidative thin-layer flow cell with a TL-5 glassy carbon working electrode and Ag/AgCl reference electrode (Merck, Darmstadt, F.R.G.), Hibar LiChrocart 125-4 Superspher 100 CH-18 reversed-phase chromatographic column, Hibar LiChrocart 25-4 LiChrosorb RP-18 guard column and Varian CDS 401 (Vista series) recorder.

The mobile phase consisted of 0.15 *M* monochloroacetate buffer (pH 3.0) containing 2 m*M* EDTA and 120 mg/l sodium octyl sulfate and was pumped at 1.3 ml/min.

The reagents included 3,4-dihydroxybenzylamine hydrobromide, norepinephrine bitartrate, epinephrine bitartrate, dopamine hydrochloride (DA), 3,4-dihydroxyphenylalanine (DOPA), monochloroacetic acid (MCAA), and Tris base (T-1503), and all were purchased from Sigma (St. Louis, MO, U.S.A.). α-Methyldopa (αMeDOPA) was obtained from Merck (Rahway, NJ, U.S.A.). Sodium octylsulfate (SOS) and acid-washed aluminum oxide (AAO) were purchased from Bioanalytical Systems (Lafayette, IN, U.S.A.).

Assay procedure

Pooled blood from black bedouin goats and healthy volunteers was collected into heparinized syringes and centrifuged at 3000 *g* for 10 min at 4°C. The samples were stored at −80°C until assayed as previously described [7]. Briefly, to each 2 ml plasma were added 50 mg activated aluminum oxide, 1 ml Tris buffer, and 25 μl internal standard, either DHBA or αMeDOPA. The sample was shaken vigorously for 5 min, the supernatant removed, and the alumina washed twice with distilled demineralized water. The catechols were desorbed by addition of 200 μl of 0.1 *M* perchloric acid, waiting 5 min, and vortex-mixing the sample. After centrifugation for 1 min, 100 μl of the supernatant were injected into the LC–ED system at room temperature. All assays were performed in duplicate.

For deproteinization of the plasma [8], 1.125 ml of 1 *M* trichloroacetic acid (TCA) was added to 5 ml plasma and the sample centrifuged for 10 min at 10 000 *g*. The supernatant was treated with 0.125 ml of 5% potassium hydroxide and recentrifuged.

The effects of dilution of the plasma with phosphate buffer (0.1 *M*, pH 7) and of incubation at 4°C for 1 h after addition of the internal standard also were assessed.

RESULTS AND DISCUSSION

As indicated in Table I, recovery of DHBA from bedouin goat plasma was less than that from human plasma. Dilution of the goat plasma with phosphate buffer increased the recovery of DHBA, whereas incubation of goat plasma for 1 h after addition of DHBA resulted in virtually no DHBA recovery. Neither sample di-

TABLE I

INFLUENCE OF PLASMA DILUTION AND INCUBATION TIME BEFORE EXTRACTION ON RECOVERIES OF INTERNAL STANDARDS

Plasma (ml)	Buffer (ml)	Time (h)	Recovery (%)					
			Human			Goat		
			DHBA	DA	αMeDOPA	DHBA	DA	αMeDOPA
2.0	0.0		54.7	63.1	28.6	9.4	43.3	28.1
1.0	1.0		56.6	59.1	32.7	18.9	45.9	30.6
0.5	1.5		58.5	60.8	32.0	37.7	53.2	31.2
0.0	2.0		56.0	55.9	30.3	56.4	52.5	30.6
		0	56.1	57.4	26.5	12.2	47.6	32.6
		1	53.8	60.2	28.5	0.0	19.6	34.7

TABLE II

INFLUENCE OF DEPROTEINIZATION ON RECOVERIES OF INTERNAL STANDARDS

Substance	Recovery (%)			
	Human		Goat	
	Before	After	Before	After
DHBA	55.0	59.7	11.3	54.6
αMeDOPA	24.3	25.8	26.3	26.8
DA	56.6	65.2	48.0	70.5

lution nor plasma incubation for 1 h affected recovery of DHBA from human plasma.

Table II shows that deproteinization of goat plasma using TCA increased the recovery of DHBA, whereas deproteinization of human plasma did not affect the recovery of DHBA.

The recovery of DA added to goat plasma also was less than that of DA added to human plasma. The DA recovery was increased by sample dilution, immediate assay after addition of the DA, and deproteinization in goat but not in human plasma (Tables I and II and Fig. 1).

When αMeDOPA was used as an internal standard, the analytical recovery was somewhat lower than that of the catecholamines, as previously reported for DOPA [9]. Recoveries of αMeDOPA were similar in goat and human plasma, and dilution of the sample, incubation for 1 h after addition of the αMeDOPA, and deproteinization did not affect its recovery.

The results demonstrate that analytical recoveries of DA and DOPA as well as DHBA can be low compared to those of NE and E in goat plasma, and the recoveries can be increased when the sample is deproteinized. The results are consis-

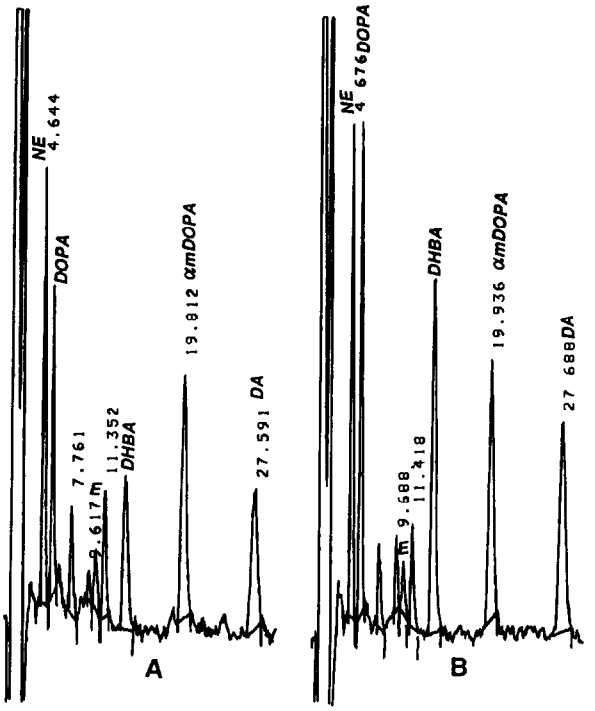

Fig. 1. Chromatographic recordings of bedouin goat plasma to which αMeDOPA, DA, and DHBA had been added (A) without deproteinization of the plasma before assay and (B) with deproteinization. Note that the recovery of DHBA, DA, and DOPA increased with deproteinization, whereas the recovery of αMeDOPA, NE, and E did not.

tent with the view that DHBA, DA, and DOPA are bound to a plasma protein in goat to a greater extent than are αMeDOPA, NE, and E.

This phenomenon is not apparent in human plasma. Previous studies have reported that albumin [10,11] and various globulins [12,13] are responsible for binding of catecholamines in human plasma. In one study [14], E was reported to be about 20% protein-bound and NE and DOPA 13% each. A recent study using equilibrium dialysis indicated that saturable binding of plasma catecholamines is mainly to α-1 acid glycoprotein and to a lesser extent to albumin or lipoproteins [15]. Simple immunoelectrophoresis has not identified important differences in patterns obtained in humans and goats (unpublished observation).

Substitution of αMeDOPA for DHBA or inclusion of a deproteinization step if DHBA is used as internal standard improves the validity of the measurements in assays of plasma catecholamines in bedouin goats.

ACKNOWLEDGEMENT

We wish to thank Mr. David Levanon for his technical assistance.

REFERENCES

1 J.M. Boylan and J.B. Susa, J. Chromatogr., 338 (1985) 192.
2 N.W. Robie and K.S. DuSapin, J. Chromatogr., 270 (1983) 366.
3 G.E. Hardee, J.W. Lai, S.D. Semrad and C.M. Trim, J. Vet. Pharmacol. Ther., 5 (1982) 279.
4 E. Maltz, K. Olsson, S.M. Glick, F. Fyhrquist, N. Silanikove, I. Choshniak and A. Shkolnik, Comp. Biochem. Physiol., 77A (1984) 79.
5 C. Wittenberg, I. Choshniak, A. Shkolnik, K. Thurau and J.B. Rosenfeld, Pflugers Arch., 406 (1986) 405.
6 K. Olsson, E. Maltz, S.M. Glick, F. Fyhrquist and A. Shkolnik, Acta Physiol. Scand., 118 (1983) 297.
7 LCEC Application Note No. 14, Bioanalytical Systems, West Lafayette, IN.
8 T. Ishimitsu and S. Hirose, J. Chromatogr., 337 (1985) 239.
9 D.S. Goldstein, R. Stull, R. Zimlichman, P.D. Levinson, H. Smith and H.R. Keiser, Clin. Chem., 30 (1984) 815.
10 A. Danon and J.D. Sapira, J. Pharm. Exp. Ther., 182 (1972) 295.
11 H.N. Antoniades, A. Goldfien, S. Zileli and F. Elmadjian, Proc. Soc. Exp. Biol. N.Y., 97 (1958) 11.
12 G. Powis, Biochem. Pharmcol., 24 (1975) 707.
13 B.L. Mirkin, D.M. Brown and R.A. Ulsrom, Nature (London), 212 (1966) 1270.
14 G. Franksson and E. Anggard, Acta Pharmacol. Toxicol., 28 (1970) 209.
15 G. Sager, H. Bratlid and C. Little, Biochem. Pharmacol., 36 (1987) 3607.