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Note

Determination of adult sheep plasma catecholamines using [^{3}H]norepinephrine as the internal standard

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Recent publications have described the use of liquid chromatography with electrochemical detection for the determination of plasma catecholamines [1, 2]. All are based on a liquid—solid extraction of the catecholamines onto alumina, followed by elution with dilute perchloric acid which serves as the injectable extract. An internal standard, 3,4-dihydroxybenzylamine (DHBA), is added to each plasma sample to be extracted. We have found the method to work well with plasma from humans, monkeys, pigs, rats, and fetal sheep. However, when neonatal or adult sheep plasma is extracted, the recovery of the DHBA is very low. This has also been reported to be a problem when extracting dog plasma [3]. We describe below a technique using [^{3}H]norepinephrine ([^{3}H]NE) as the internal standard to calculate the recovery of catecholamines in neonatal and adult sheep.

MATERIALS AND METHODS

Sample extraction was run in 1.5-ml polypropylene tubes to which the following had been added: 50 μl of 5 m*M* sodium hydrogen sulfite, 50 μl of 0.1 *M* perchloric acid or 50 μl of [^{3}H]NE (New England Nuclear, diluted to 0.0033 μCi per 50 μl of 0.1 *M* perchloric acid) and 50 μl of DHBA at a concentration of 1 ng per 50 μl of 0.1 *M* perchloric acid.

Differing amounts of pooled adult sheep plasma were then added. When using less than 1 ml of plasma, the volume was brought up to 1 ml with 0.1 *M* phosphate buffer, pH 7.0. Next, 20 mg of aluminum oxide (Fisher, activated by the method of Anton and Sayre [4]) were added to each tube followed by 600 μl of 1.5 *M* Tris buffer. The sample was then vigorously shaken for 15 min,

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the supernatant aspirated and the alumina washed three times with distilled water. After the final washing the alumina was aspirated dry. Next, 200 μl of 0.2 *M* perchloric acid were added to the alumina, the tubes shaken for 15 min, then centrifuged. A 100-μl aliquot of the perchloric acid extract was then injected into the HPLC system. In the samples where [^{3}H]NE was used as the internal standard, 50 μl of the perchloric acid extract were also counted in 5 ml of Aquasol with a Packard Tri-Carb liquid scintillation counter.

The high-performance liquid chromatograph consisted of an M-45 dual-piston pump (Waters Assoc.), a WISP 710B automated sample injector (Waters Assoc.), a 25-cm ODS 5-μm column (IBM Instruments), a glassy carbon working electrode with an Ag/AgCl reference electrode (BioAnalytical Systems), an LC-4B amperometric detector and an LC-22A temperature controller (BioAnalytical Systems), and an M730 Data Module (Waters Assoc.).

The mobile phase contained 100 m*M* sodium phosphate monobasic, 2.75 m*M* octanesulfonic acid, sodium salt (Eastman Kodak), 0.1 m*M* EDTA, disodium salt, and 5.5% acetonitrile. The flow-rate was 1.2 ml/min at 30°C with a working electrode potential of 0.65 V and a sensitivity of 0.5 nA.

RESULTS AND DISCUSSION

Our laboratory recently began measuring catecholamines in sheep plasma using the same methodology as that for human plasma. This method had already been successfully used for measuring catecholamines in monkeys, pigs, and rats with DHBA recoveries of 70—80%. It was observed that as seen with human plasma, 1 ml of fetal sheep plasma had a DHBA recovery of 70—80%. However, adult sheep showed a much lower recovery. We found that as the

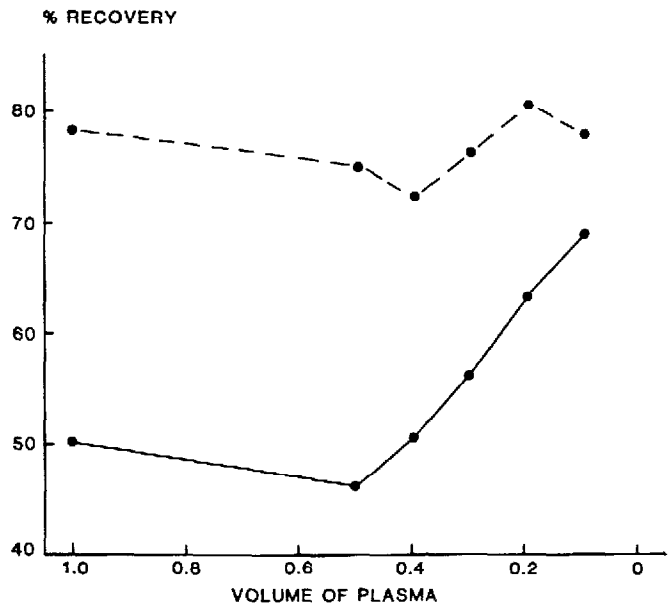

Fig. 1. Percent recovery of DHBA (—) and [^{3}H]NE (- - -) from differing volumes of adult sheep plasma.

TABLE I

CALCULATED VALUES FOR POOLED ADULT SHEEP PLASMA NE

Amount of plasma extracted (ml)	Recovery of DHBA (%)	NE using DHBA recovery (pg/ml)	Recovery of [^{3}H]NE (%)	NE using [^{3}H]NE recovery (pg/ml)
1.0	50.2	2364	78.2	1519
0.5	46.2	2312	75.2	1412
0.4	50.4	2275	72.4	1584
0.3	56.2	1667	76.3	1227
0.2	63.3	1815	80.7	1424
0.1	68.9	1580	78.0	1398

amount of adult sheep plasma used in the extraction procedure decreased, the recovery of DHBA increased (Fig. 1). The peak heights of NE, when corrected for the amount of plasma extracted, were comparable, showing NE recovery was not effected. The variation in DHBA recovery caused the calculated values of NE to be erroneously high (Table I).

Because we found the recovery of NE not to be effected, we chose [^{3}H]norepinephrine as our internal standard. The extraction procedure is the same with the exception of [^{3}H]NE which was diluted in 0.1 *M* perchloric acid being substituted for 0.1 *M* perchloric acid alone. NE values were calculated from the chromatogram, subtracting the amount of NE added, then using the recovery of [^{3}H]NE (Table I).

The reason for the low recovery of DHBA is not known but may be, as speculated by Robie and DuSapin [3], the result of protein binding which does not occur with NE. In sheep this protein appears in the plasma shortly after birth since the recovery of DHBA in fetal sheep is high.

In summary, when measuring catecholamines in neonatal or adult sheep, an internal standard other than DHBA must be used because the DHBA recovery by alumina extraction is low as compared to that of NE giving incorrect high values for NE. We have found [^{3}H]NE to be a satisfactory substitute.

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