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NON-RADIOCHEMICAL PROCEDURE FOR THE MEASUREMENT OF O-METHYLATION OF THE STEREOISOMERS OF ISOPRENALINE

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SUMMARY

A non-radiochemical procedure has been developed which permits the separation and measurement of isoprenaline (ISO) and the O-methylated metabolite, 3-methoxyisoprenaline (MeOISO). This methodology employs alumina chromatography and toluene solvent extraction to separate the catecholamine, ISO, from the O-methylated derivative, MeOISO. High-performance liquid chromatography with electrochemical detection has been used to quantify these compounds. The biological application of this procedure includes the evaluation of O-methylation of the stereoisomers of ISO by intact tissues.

INTRODUCTION

Extraneuronal uptake (ENU) and O-methylation by the enzyme catechol-O-methyltransferase (COMT) are mechanisms for the synaptic removal and intracellular inactivation of the neuronally released transmitter, noradrenaline, by effector tissues. Isoprenaline (ISO) has been used commonly as a substrate for examining these two processes. Since this catecholamine is not metabolized by the enzyme monoamine oxidase, but is metabolized by COMT it has only one major metabolite, 3-methoxyisoprenaline (MeOISO). Additionally, ISO is also not a good substrate for neuronal uptake. Thus, this amine appears to be an ideal substrate for studies on ENU since its uptake and metabolite profile is less complicated than that for other catecholamines including the naturally occurring noradrenaline and adrenaline.

Much of the experimentation in this area has involved the use of tritiated *dl*-isoprenaline ([^{3}H]ISO). The methods used in the past for the separation of [^{3}H]ISO and [^{3}H]MeOISO have included thin-layer chromatography [1], solvent extraction [2], and column chromatography [3]. The limitations of

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these experimental approaches lie not within the methodologies themselves, but in the use of radiolabeled substrates. In addition to the high cost of purchase and disposal of radiochemicals, several laboratories have described the presence of impurities as a problem with several substrates including ^{3}H-ISO [2, 4]. Impurities create the additional requirement of employing a purification procedure prior to the use of the radiolabeled substrate.

Although radiochemical procedures are quite sensitive, their use in studies of ENU are severely limited. For example, an examination of the stereospecificity of the ENU system using ISO as substrate cannot be accomplished since ^{3}H-ISO is commercially available only as the racemic mixture. Another drawback associated with the use of radiochemical procedures is the difficulty encountered in attempting to carry out multi-substrate studies. This is due both to the limitations of scintillation spectrometry and to the cross-contamination that can occur when different isotopically labeled compounds are used.

The problems outlined about have prompted us to seek a non-radiochemical procedure for the detection of ISO and MeOISO which approaches the sensitivity of the existing radiochemical methods. Most of the existing non-radiochemical procedures which employ high-performance liquid chromatography (HPLC) to examine O-methylation and COMT activity involve the use of purified rat liver COMT with other catecholamines such as noradrenaline or adrenaline as substrates [5—7]. Since the primary aim of our study was to examine the uptake and O-methylation of ISO by intact vascular tissue, these procedures could not be employed. We present here a newly developed assay procedure employing HPLC with electrochemical detection (ED) that permits the measurement of the O-methylation of ISO which occurs in intact vascular tissue.

EXPERIMENTAL

Apparatus

An HPLC apparatus consisting of a Waters Assoc. M-45 pump solvent delivery system, a Waters U6K universal injector with 1.5-ml injection sample loop, C_{18} filled guard and 10 μBondapak C_{18} stainless-steel reversed-phase columns (30 cm × 3.9 mm I.D.), and radial compression cartridges prepacked with C_{18} (10 μm, 12 cm × 8 mm I.D.) were used for the separation procedure. A Bioanalytical LC-4B amperometric detector with a glassy carbon electrode was used for the electrochemical detection measurements.

Chemicals

d-ISO (bitartrate salt), *l*-ISO (hydrochloride), and 3-methoxytyramine were obtained from Sigma (St. Louis, MO, U.S.A.). 3,4-Dihydroxybenzylamine (hydrobromide) was obtained from Aldrich (Milwaukee, WI, U.S.A.). 3-Methoxyisoprenaline and 3,4-dihydroxy-2-methyl propriophenone were gifts of Boehringer Ingleheim (Ridgefield, CT, U.S.A.) and Upjohn (Kalamazoo, MI, U.S.A.), respectively. Acid alumina AG4 was purchased from Bio-Rad Labs. (Richmond, CA, U.S.A.) HPLC grade methanol, EDTA and acids and salts for the various buffers were reagent grade from Fisher Scientific (Fair Lawn, NJ, U.S.A.).

Incubation procedure

The tissue incubation procedure employed was a modification of that described by Levin [8]. Male New Zealand white rabbits (1.5—3.5 kg) were killed by stunning with a blow to the head followed by exsanguination. The entire length of the thoracic aorta (from heart to diaphragm) was removed and cleaned of adhering blood and fat, slit lengthwise, cut into eight segments, blotted and weighed (25—55 mg segments). The segments were then placed in 1 ml of physiological Krebs solution (117 m*M* NaCl, 4.8 m*M* KCl, 1.2 m*M* KH_2PO_4, 25 m*M* $NaHCO_3$, 2.5 m*M* $CaCl_2$, 5.5 m*M* glucose, 0.03 m*M* EDTA), and oxygenated with oxygen—carbon dioxide (95:5) at 37° C. The tissues were preincubated for 30 min prior to transfer to 1 ml of fresh oxygenated Krebs solution. ISO was then added and the tissues, along with appropriate drug blanks, tissue blanks, and recovery tubes, were incubated for an additional 60 min.

Post-incubation tissue preparation

After the 1-h incubation period, the tissue segments were washed for 1 min in fresh oxygenated Krebs solution, blotted, and placed in 1 ml of 0.4 *M* perchloric acid containing the appropriate internal standards. Dihydroxybenzylamine (DHBA) and 3-methoxytyramine (3-MT) were the internal standards for ISO and MeOISO, respectively. The concentration of internal standard used varied with the incubation substrate concentration. The tissues were then homogenized using a glass—glass homogenizer (Kontes Duall size 20) equipped with a motor-driven pestle. The homogenate was placed in a 1.5-ml capacity polypropylene microcentrifuge tube and centrifuged for 5 min at 3250 *g* (Beckman Microfuge II). The resulting supernatant fluid was then frozen (—5° C) until a precolumn extraction could be performed.

Post-incubation incubate preparation

After the 1-h incubation, 800 μl of the incubation solution were added to tubes containing the appropriate internal standards in 100 μl of 4.0 *M* perchloric acid. The internal standards were made in 0.01 *M* hydrochloric acid, and then diluted to the proper concentration in 4.0 *M* perchloric acid shortly before adding the incubation solution. A 100-μl aliquot of fresh Krebs solution was added to give a final volume of 1 ml and a final perchloric acid concentration of 0.4 *M*. The samples were then vortexed and frozen to await precolumn extraction. It should be noted that a correction factor of 1.25 was applied when calculating the total incubate metabolite concentrations in order to account for the entire 1 ml of original incubation solution.

Precolumn purification

The precolumn purification procedure was designed to separate the substrate, ISO, and the metabolite, MeOISO, into separate fractions.

Catechol fraction (ISO)

The frozen acidified samples of incubation media and/or tissue homogenate extracts were thawed and 200 μl of a sodium metabisulfate—EDTA mixture (0.5 g sodium metabisulfite in 20 ml of 5% EDTA solution) were added; acid alumina (140 mg) also was added. The tubes were vortexed and the pH

adjusted to 8.4 with 3 *M* Tris buffer (pH 10.9). The tubes were shaken (approximately 220 oscillations per min) at a 45° angle for 15 min, and then centrifuged for 2 min at 140 *g*. The resulting supernatant fluid (alumina effluent) was collected and stored on ice for later processing (see *O-Methylated catechol fraction*). The alumina was washed with 5 ml of 0.03 *M* phosphate buffer (pH 7.3) by vortexing and centrifuging; wash solutions were discarded. The wash procedure was then repeated with 3.0 ml of distilled water.

Catechols were eluted from the alumina by washing twice with 300 μl of 1 *M* acetic acid containing 0.01 *M* boric acid. Each elution was carried out by shaking the samples for 10 min and then centrifuging. The eluates were pooled, placed in glass culture tubes, and dried on a Buchler vortex evaporator (approximately 1 h). Once dried, the samples were reconstituted in 200 μl of 0.05 *M* acetic acid and frozen until assay by HPLC.

O-Methylated catechol fraction (MeOISO)

To the alumina effluent (pH 8.4) that was saved from a previous step (see above), 250 μl of sodium borate (2 *M* sodium hydroxide with 1 *M* boric acid) were added. Powdered potassium phosphate (dibasic, anhydrous, 2.5 g) was then added and the samples were vortexed. Toluene (5 ml) was added and the samples were shaken for 15 min. Following centrifugation (140 *g* for 2 min), 4 ml of the toluene phase were transferred to tubes containing 500 μl of 0.5 *M* acetic acid. Fresh toluene (5 ml) was added to the original tubes and the extraction steps (i.e., shaking, centrifugation, and 4.0 ml transfer) were repeated. The resulting 8.5 ml of toluene and the acetic acid were then shaken for 20 min. Following centrifugation, the aqueous layer was frozen (dry ice—methanol) and the toluene layer discarded. The aqueous layer was then thawed and transferred to glass culture tubes and dried in a Buchler vortex evaporator for approximately 1 h. The dried samples were reconstituted in 200 μl of 0.05 *M* acetic acid and were frozen awaiting HPLC assay. It should be noted that a correction factor of 1.25 had to be used when calculating the total O-methylated catecholamine content to account for the entire 10 ml of toluene used in the extraction process.

HPLC—ED assay

Conditions for ISO and DHBA. A Waters reversed-phase C_{18} radial compression module cartridge (12 cm × 8 mm I.D.) was used for the separation of ISO and DHBA. The mobile phase consisted of a citrate—phosphate buffer (0.1 *M* $Na_2HPO_4 \cdot 7H_2O$ with the pH adjusted to 4.0 with 0.1 *M* citrate solution) containing 25—35% methanol. The amount of methanol used was dependent upon the age and amine retention times of individual cartridges. The mobile phase was delivered by the pump at a flow-rate of 2 ml/min. The potential setting which gave optimal detection was 0.70 V.

Conditions for MeOISO and 3-MT. A Waters reversed-phase stainless-steel C_{18} μBondapak (300 mm × 3.9 mm I.D.) column was used for the separation of MeOISO and 3-MT. The citrate—phosphate buffer (pH 4.0) containing 15% methanol served as the mobile phase. The pumping flow-rate of the mobile phase delivery system was 1 ml/min. The optimal detection was achieved at a potential setting of 0.80 V.

Synthesis of d-MeOISO and l-MeOISO. The synthesis of *d*- and *l*-MeOISO was a modification of that of Head et al. [2]. *d*- or *l*-ISO was incubated in the presence of the cofactors S-adenosylmethionine, magnesium chloride and buffered purified rat liver COMT; the enzyme was prepared according to the method of Sole and Hussian [9]. The enzymatic O-methylation reaction was stopped after a 1-h incubation by adding tetraphenyl borate and sodium borate. The *d*- or *l*-MeOISO was then extracted with toluene—isoamyl alcohol and back-extracted over acetic acid. The acid was dried and the samples were reconstituted in an appropriate volume of 0.05 *M* acetic acid prior to analysis by HPLC—ED.

RESULTS AND DISCUSSION

Chromatographic properties

Preliminary studies, using an isocratic solvent system, have shown that ISO and MeOISO can be identified by electrochemical detection and that these substances have reversed-phase column retention times greater than those of the endogenous catecholamines. The prolonged retention times eliminate possible problems of interference by the endogenous catecholamines. However, our results also demonstrated that a simple isocratic solvent system is not altogether suitable for the determination of both ISO and MeOISO in a single sample when employing our method since there was a considerable difference in the retention times between the two compounds. A gradient elution technique could not be considered since drastic shifts in the ED baseline occurred when this form of elution was attempted.

Experiments were caried out with a variety of solvent systems, using both stainless-steel columns and radial compression module cartridges. Two separate conditions (see Experimental), one for ISO and the other for MeOISO, were developed. When these conditions were used, the retention times for the respective isomers of each amine (i.e., *d*- and *l*-ISO approximately 6 min; *d*- and *l*-MeOISO 7 min) were identical. *d*-MeOISO and *l*-MeOISO were synthesized biologically in our laboratory since they were not commercially available as separate compounds (see Experimental). This was done because it was considered important to verify that the retention times and electrochemical properties of the two isomers were identical. This finding assured us that the racemic mixture could be used as the standard for the HPLC—ED assay.

To achieve maximum sensitivity with minimum background current, it was necessary to establish a voltage—response curve for each compound of interest, i.e. *d*- and *l*-ISO, *dl*-, *l*- and *d*-MeOISO. The voltage—response curves for the *d*- and *l*-isomers of ISO are identical (Fig. 1) as were those for the electrochemical measurement of *d*- and *l*-MeOISO (Fig. 2). From the curves in Figs. 1 and 2, it was determined that 0.7 V was the optimal working potential for ISO while 0.8 V was optimal for MeOISO.

Linearity of response of the HPLC—ED assay

After the optimal chromatographic and electrochemical conditions were developed for ISO and MeOISO, it was necessary to determine the linearity of the HPLC—ED response. A range of 1 ng to 1 μg was chosen since studies

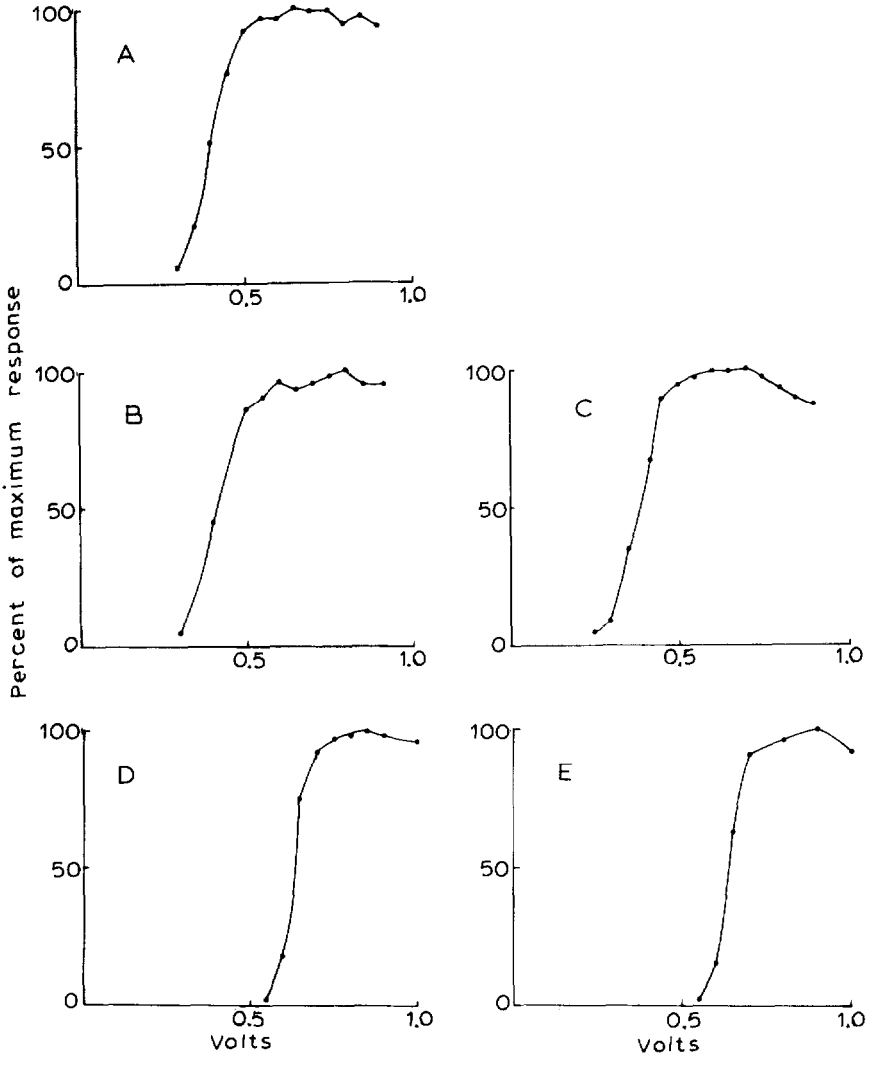

Fig. 1. Voltage—response curves constructed by using a standard concentration of *l*-ISO (A), *d*-ISO (B), DHBA (C), *dl*-MeOISO (D) and 3-MT (E) at various voltage settings.

reported in the literature indicated that this was an appropriate concentration range to use for studies of ENU. The data demonstrate that the responses to increasing amounts of ISO and MeOISO were linear from 1 ng to 1 μg with a correlation coefficient of 0.99. Furthermore, the lower limit of detectability was calculated to be approximately 200 pg.

Precolumn purification

The use of a precolumn purification procedure for the separation of ISO and MeOISO from biological samples was indicated for several reasons. First, our original isocratic HPLC—ED assay could not be used to analyze ISO and MeOISO from a single sample due to the rather large differences in their retention times. A single sample analysis of ISO and MeOISO was also

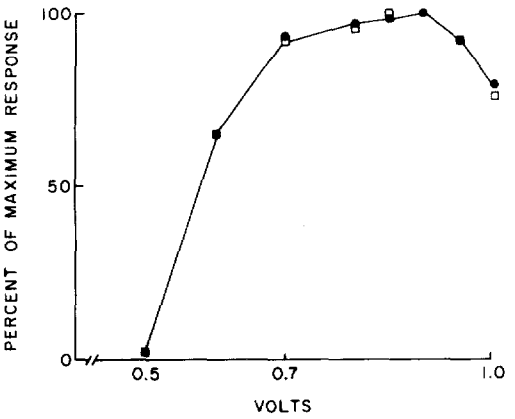

Fig. 2. Voltage—response curves constructed using *d*-MeOISO (•) and *l*-MeOISO (□) synthesized in the laboratory. The response of a standard volume of drug was measured at various voltage settings.

unwarranted due to the difference in concentration of ISO and MeOISO that occurred after a 1-h incubation period. Therefore, a method had to be developed to isolate and separate the ISO from MeOISO prior to HPLC—ED assay. Secondly, since the incubation and extraction solutions obtained after the tissue incubations have a relatively large volume, they are not suitable for direct analysis. The Krebs incubation solutions have a volume of 1 ml and have a considerable inorganic ion content. In addition, the perchloric acid extracts of the tissues also are 1 ml in volume and are extremely acidic in nature. Thus, a precolumn preparation offers a method by which purification and volume—concentration adjustment can be obtained in addition to the separation of ISO from its O-methylated metabolite.

Precolumn isolation of ISO

It has been known for some time that catechol compounds bind to acidic alumina in an alkaline medium. It is also known that the catecholamine compounds are readily oxidized under such high pH conditions, and that it is necessary to add an antioxidant to the tissue acid extracts and the acidified incubation solutions before increasing the pH for the alumina extraction. The antioxidant sodium metabisulfite (NaMBS) in an EDTA solution was chosen. NaMBS is a potent, non-catechol, non-acidic antioxidant that will not interfere or bind to the alumina at basic pH values. Since metal ions can promote the oxidation process, a metal ion chelator, EDTA, also was added prior to alumina extraction. A 3 *M* Tris buffer (pH 10.9) was used to increase the pH of the solutions after the addition of the antioxidant solution and alumina. It was found that a pH range of 8.2—8.4 was critical to ensure maximal catechol—alumina binding. The pH of each sample was individually adjusted during the addition of the Tris buffer. It was also necessary to vortex the sample while adding Tris to ensure consistency of pH throughout final solution.

ISO was removed from the alumina through use of two separate elutions (300 μl each) with 1 *M* acetic acid. The final volume (600 μl), although less than that of the original post-incubation solution, was still considered too large and too strongly acidic for optimal HPLC—ED assay. An additional step in

which the samples were taken to dryness on a Buchler vortex evaporator was added. This permitted the readjustment of final volume in a suitable vehicle solution. It was of concern that during the drying process the catechols may be oxidizing as the acid volume decreased. To avoid the possible oxidation of these compounds boric acid was added to the 1 *M* acetic acid used in the back-extraction. Boric acid binds to catechol groups in alkaline conditions, thus preventing their oxidation. Several concentrations of borate were tested (0.2 an 0.01 *M* borate in 1 *M* acetic acid). Unfortunately, there was a considerable amount of salt residue found after drying in those tubes which contained the highest borate concentration. Some of this residue was lost as the vacuum of the evaporator was turned off (the change in pressure pushed the salt up and out of the tubes, especially when vortexing was vigorous). By using the lower borate concentration, the problem was avoided and the recoveries were improved.

Several acid solutions were examined for use as a sample reconstituting medium (0.04 *M* perchloric acid, 0.05 *M* acetic acid). When assayed on HPLC—ED, blank samples reconstituted in perchloric acid contained a number of peaks, several of which interfered with the peaks of interest. 0.05 *M* Acetic acid was chosen as the reconstitution acid since it was not corrosive and produced no interfering peaks.

Precolumn isolation of MeOISO

Preliminary studies in this laboratory have shown that O-methylated compounds, such as normetanephrine and metanephrine, can be extracted readily from an alkaline medium with chloroform once the aqueous phase was saturated with K_2HPO_4 (2.5 g). Unfortunately, chloroform was found to be unreliable for the extraction of MeOISO from the alkaline effluent. Several solvents and solvent combinations possessing a range of polarities were tested for their ability to extract MeOISO from the salt-saturated alkaline medium. A summary of these results are given in Table I. Toluene, a relatively non-polar solvent, gave the best recovery for MeOISO and provided the best correlation between the recoveries of MeOISO and its internal standard, 3-methoxytyramine (3-MT) (internal standards will be discussed in the next section).

TABLE I

SOLVENTS TESTED FOR MeOISO EXTRACTION

Values expressed are the mean ± standard error, $n = 3$.

Solvent	Percentage recovery*	
	MeOISO	3-MT
Chloroform	49.3 ± 8.8	63.3 ± 3.3
Chloroform + 25% ethyl acetate	37.7 ± 1.7	55.3 ± 0.3
Chloroform + 25% heptane	51.0 ± 7.7	63.0 ± 3.0
Methylene chloride	28.7 ± 5.7	23.0 ± 3.0
Toluene	68.7 ± 6.4	69.3 ± 4.6

*Percentage recovery after extraction of standard concentration (1 μg) MeOISO, 0.5 μg 3-MT.

Since toluene rather than chloroform was to be used in the present studies, it was necessary to reexamine the salt conditions used previously when extracting with chloroform (i.e. 2.5 g K_2HPO_4). Differing amounts of potassium phosphate (1.0, 2.0, 2.5, 3.0 g) and sodium chloride (1.0, 2.0, 2.5 g), were tested for their effect on the toluene extraction of MeOISO from the aqueous alkaline medium. The best recovery of MeOISO was obtained when K_2HPO_4 was used, with additions of 2.5 g and 3.0 g giving similar consistent results; 2.5 g (the same amount as that used with chloroform) was chosen.

Two successive 5-ml portions of toluene were used to extract the O-methylated MeOISO from the aqueous salt-saturated phase. The two aliquots were then pooled over 500 μl acid (see below) for back-extraction. During the removal of the 10 ml of solvent (toluene) in the extraction process (i.e., two lots of 5 ml), some the aqueous salt layer was occasionally transferred to the back-extraction acid tube. This seemed to interfere with the back-extraction of MeOISO into the acid. To avoid this problem, 4-ml aliquots of each 5-ml portion of toluene were collected and pooled. A correction factor of 1.25 was then applied to the data.

When 0.1 *M* hydrochloric acid was used in the back-extraction of MeOISO in the preliminary studies, a lower recovery rate and additional peaks on the chromatogram began to appear. A similar decomposition of amines in hydrochloric acid has been documented recently by Head et al. [10]. In view of this breakdown, several concentrations (1 *M*, 0.5 *M*, 0.1 *M*) of acetic acid, an organic rather than a mineral acid, were tested for their effectiveness in back-extraction. 0.5 *M* Acetic acid gave consistent results for the extraction of MeOISO. Since the volume (500 μl) of acid used for the back-extraction was relatively large, it became necessary to dry the samples. The same conditions for drying and reconstitution used for ISO (see above) were employed for MeOISO.

Internal standards

Extraction and separation procedures are subject to loss of compound. To correct for this potential problem, the use of internal standards has been examined. Since we have found that there is no loss of compounds during the incubation, we proceeded to test the next phase of extraction procedure, i.e., experiments were conducted in which standard concentrations of ISO and MeOISO were dried in the vortex evaporator. The results indicated that there was no loss during the drying process. However, further analysis of the overall experimental method suggested that incomplete extraction was still a problem. There was a decrease in the recovery of ISO during the alumina extraction and a decrease in MeOISO recovery during the toluene extraction because of incomplete solvent and/or acid extraction. Since each of these losses occur in different fractions, two internal standards had to be employed.

Isoprenaline internal standard

Various compounds were tested as possible internal standards for ISO. Since the precolumn isolation and purification of ISO involves alumina extraction, catechol compounds capable of being adsorbed to alumina were examined. The catechol compounds tested were: epinine, dopamine, α-methylnoradrenaline,

TABLE II

PERCENTAGE RECOVERY FOLLOWING ALUMINA ABSORPTION AND ELUTION

Values represent mean ± standard error; $n = 8$.

	Recovery (%)
Isoprenaline	42.4 ± 1.8
DHBA	46.6 ± 2.0

2-methyl-3-(3,4-dihydroxyphenylalanine), α-methyldopamine, 3,4-dihydroxyphenylethyleneglycol, and dihydroxybenzylamine (DHBA). DHBA appeared to be the best candidate to serve as an internal standard for ISO since it did not interfere with the electrochemical detection of ISO, and since it is not an endogenous substance. The recovery values for DHBA and ISO from alumina are shown in Table II and are clearly seen to be almost identical.

Methoxyisoprenaline internal standard

The internal standard for the O-methylated fraction, i.e., the one containing MeOISO, had to be an O-methylated catecholamine capable of being extracted

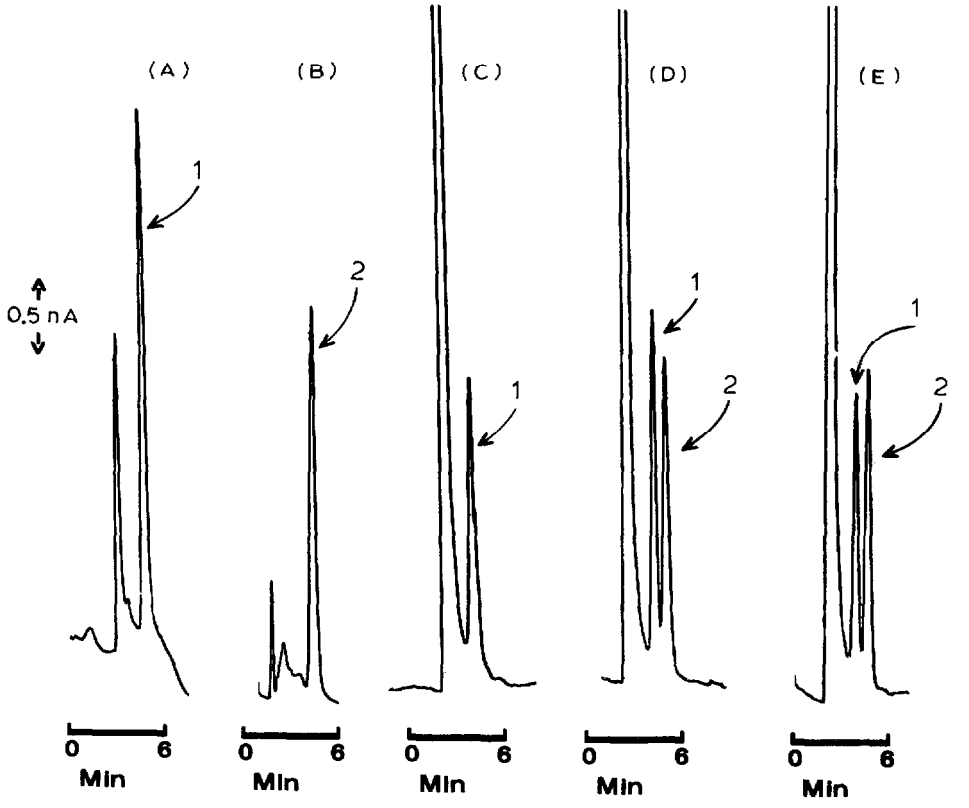

Fig. 3. Chromatograms of the content of MeOISO in the incubation solutions after a 1-h incubation period in the presence or absence of *d*- or *l*-ISO (5 μ*M*) (see Experimental for conditions employed). A, 5 ng 3-MT; B, 10 ng MeOISO; C, aorta no ISO; D, aorta *l*-ISO; E, aorta *d*-ISO. Peaks: 1 = 3-MT; 2 = MeOISO.

with toluene and also having chromatographic properties similar to MeOISO. Normetanephrine and metanephrine chromatographed too near the solvent front and since these compounds are inhibitors of extraneuronal uptake and could possibly be needed in future experiments, they were not considered. 3-Methoxytyramine (O-methylated dopamine) seemed to be a prime candidate since it chromatographed near MeOISO when the HPLC—ED conditions established previously for MeOISO were used. For a voltage—response curve for 3-MT refer to Fig. 1. The toluene extraction of 3-MT also closely followed that of MeOISO. Thus, 3-MT fit all of the criteria necessary to be an ideal internal standard for MeOISO.

Validity of total method

After the entire assay procedure (incubation, precolumn separation, and HPLC—ED assay) was developed, it was necessary to verify the methodology using segments of rabbit thoracic aorta incubated with 4.7 μM of *d*- or *l*-ISO. Sample chromatographs obtained after the extraction of incubate MeOISO, tissue MeOISO and tissue ISO are shown in Figs. 3—5. Chromatograph C on all three figures demonstrates that the ISO and MeOISO peaks were absent when the tissues were not incubated with ISO. Furthermore,there was no interference produced by any endogenous compound.

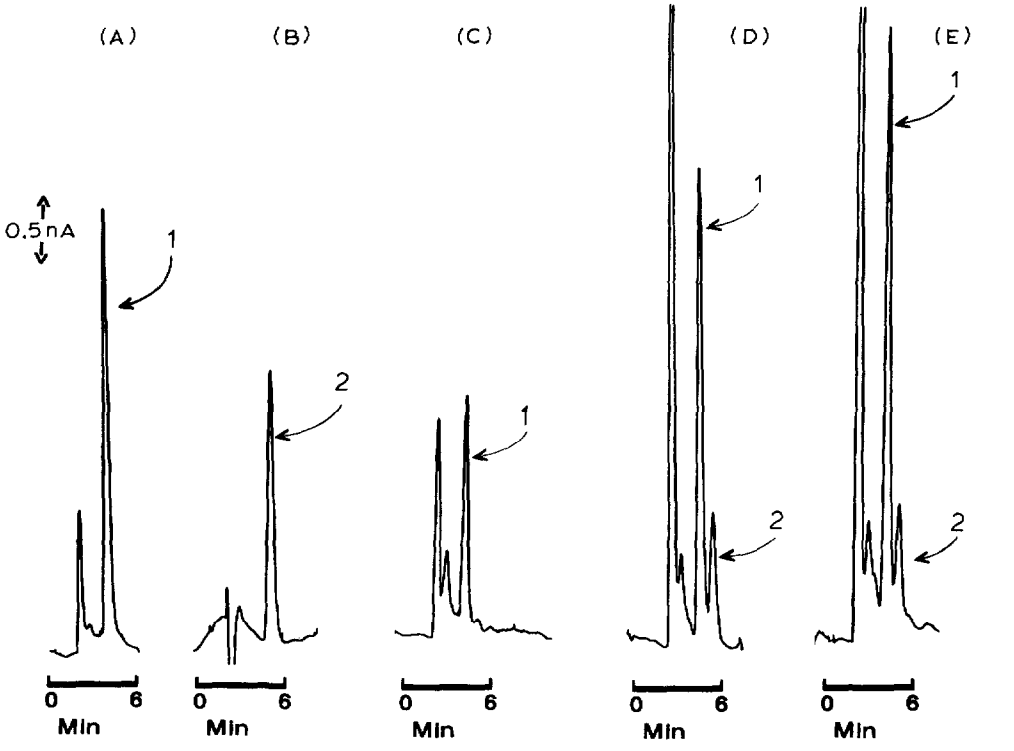

Fig. 4. Chromatograms of the content of MeOISO in the tissue following a 1-h incubation period in the presence or absence of *d*- or *l*-ISO (5 μM) (see Experimental for conditions employed). A, 5 ng 3-MT; B, 10 ng MeOISO; C, aorta no ISO; D, aorta *l*-ISO; E, aorta *d*-ISO. Peaks: 1 = 3-MT; 2 = MeOISO.

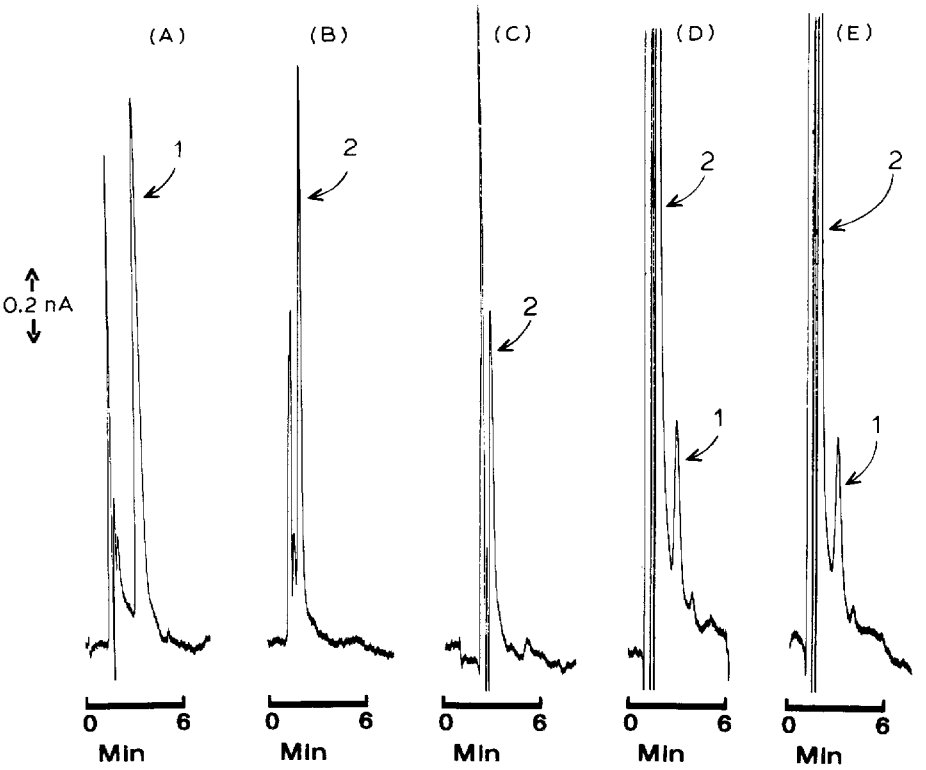

Fig. 5. Chromatograms of the content of ISO in the tissue after a 1-h incubation period in the presence or absence of *d*- or *l*-ISO (5 μM) (see Experimental for conditions employed). A, 5 ng ISO; B, 2 ng DHBA; C, aorta no ISO; D, aorta *l*-ISO; E, aorta *d*-ISO. Peaks: 1 = ISO; 2 = DHBA.

TABLE III

INCUBATE MeOISO (μg/g) AFTER INCUBATION WITH U0521 (10 μg/ml)

	n	Control	U0521
d-ISO	10	2.99 ± 0.30	< 0.30 ± 0.02*
l-ISO	9	4.11 ± 0.24	< 0.36 ± 0.03*

*$p < 0.01$.

In order to verify whether the substance found in the incubation medium and tissue after ISO incubation was actually MeOISO, the known COMT inhibitor U0521 was added prior to addition of ISO to the bath. The incubate MeOISO content after U0521 treatment decreased dramatically and fell below the level of detectability (Table III).

The results above appear to verify that the methodology developed will provide a valid approach to the study and detection of the metabolism of ISO in the intact rabbit thoracic aorta.

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REFERENCES

1 R.J. Head, R.J. Irvine and J.A. Kennedy, J. Chromatogr. Sci., 14 (1976) 578.
2 R.J. Head, I.S. de la Lande, R.J. Irvine and S.M. Johnson, Blood Vessels, 17 (1980) 229.
3 K.H. Graefe, F.J.E. Stefano and S.Z. Langer, Biochem. Pharmacol., 22 (1973) 1147.
4 P. Hjemdahl, M. Dakeskog and T. Kahan, Life Sci., 25 (1979) 131.
5 R.T. Borchardt, M.F. Hegazi and R.L. Schowen, J. Chromatogr., 152 (1978) 255.
6 M.T.I.W. Schüsler-van Hees and G.M.J. Beijersbergen van Henegouwen, J. Chromatogr., 196 (1980) 101.
7 S. Koh, M. Arai, S. Kawai and M. Okamoto, J. Chromatogr., 226 (1981) 461.
8 J. Levin, J. Pharmacol. Exp. Ther., 190 (1974) 210.
9 M. Sole and M. Hussian, Biochem. Med., 18 (1977) 301.
10 R.J. Head, J. Hemstead and B. Berkowitz, Blood Vessels, 19 (1982) 135.